

1 of 3  
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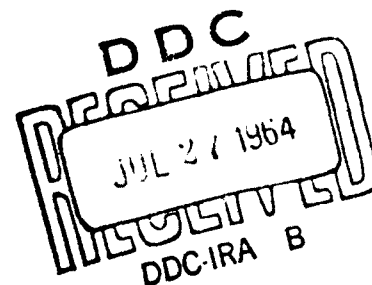
Dr. B.A. Sobin

Chas. Pfizer & Company, Inc.

Brooklyn, N.Y.

Preparation and Evaluation of Staphylococcal Enterotoxoids

Contract No. DA-49-193-MD-2428



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1 of 3

## ABSTRACT

1. Preparing Institution: Chas. Pfizer & Company, Inc.,  
Brooklyn 6, N.Y.
2. Title of Report: Preparation and Evaluation of  
Staphylococcal Enterotoxoids
3. Principal Investigator: Dr. B.A. Sobin
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↓  
Thirty Staphylococcal enterotoxoids were prepared and evaluated in rhesus monkeys in a range finding study designed to compare toxicities, antibody response, and protection against intravenous challenge with untreated Staphylococcal enterotoxin.

The immunization schedule consisted of three subcutaneous injections of toxoid with adjuvant spaced at intervals of five weeks. Some toxicity (emesis, diarrhea) was observed with most of the toxoid preparations following the first subcutaneous immunization injection. No toxic reactions followed the second and third immunization injections.

Antibody titers, as measured by hemagglutination and gel-diffusion, were generally low after the first immunization injections. Many of the antibody titers rose sharply after the second and third injections.

A number of the Staphylococcal enterotoxoid preparations provided excellent protection in monkeys against intravenous challenge with untreated Staphylococcal enterotoxin ~~at 10  $\mu$ /kg. and 1000  $\mu$ /kg.~~ three weeks after the third and final immunization injections.

↑

The ten most promising toxoid preparations were selected from the range finding studies for expanded toxicity comparisons, antibody titers, and degree and duration of immunity to intravenous challenge with Staphylococcal enterotoxin.

In the extended evaluation studies to date, Sample #10 (0.2% formalin + 0.15% glycine at 25°C. for 2 weeks) is the most impressive of the toxoid preparations on the basis of toxicity and antigenic response. The final evaluation will depend on the degree and duration of immunity to intravenous challenge with Staphylococcal enterotoxin, correlation of immunity with hemagglutination titers, effect of booster shots, and nature of anamnestic response.

7. Protection against intravenous challenge with Staphylococcal enterotoxin based on immunization with enterotoxoid preparations.

#### ABSTRACT DISTRIBUTION LIST

Dr. J.M. Beller  
The National Drug Company  
Division of Richardson-Merrell Inc.  
4663 Stenton Avenue  
Philadelphia 14, Pennsylvania

Dr. Winston H. Price  
John Hopkins University  
School of Hygiene & Public Health  
615 North Wolfe Street  
Baltimore 5, Maryland

Dr. Ronald S. Ratney  
Hood College  
Frederick, Maryland

Dr. Theodore E. Woodward  
University of Maryland  
Baltimore, Maryland

## INTRODUCTION

The establishment of the protein and antigenic nature of Staphylococcal enterotoxin, and the availability of a supply of highly purified material provided the background for the following objectives:

- a. To prepare a number of toxoids of Staphylococcal enterotoxin by treatment with a variety of chemical and physical agents.
- b. To test and evaluate the immunological responses of the various Staphylococcal enterotoxoid preparations in rhesus monkeys.
- c. To select a safe and effective Staphylococcal enterotoxoid capable of conferring upon rhesus monkeys immunity of long duration against the action of a large dose of intravenously administered Staphylococcal enterotoxin.

The construction of an animal facility to house two hundred rhesus monkeys was begun in the early part of May, 1963, and was completed August 15, 1963. Animal experimentation was initiated September 3, 1963.

Working supplies of highly purified Staphylococcal enterotoxin, Lot No. 14-31, were made available on May 28 and June 17, 1963.

A search of the literature was made covering the chemical and physical agents used to prepare toxoids, inactivate microorganisms, and alter proteins. Thirty toxoid treatments were selected for evaluation in a preliminary range finding study comparing toxicity, serologic response, and protection against intravenous challenge with untreated Staphylococcal enterotoxin. Ten Staphylococcal enterotoxoid preparations were chosen from this group for evaluation over a twelve month period with a scheduled termination date of May 30, 1965.

## MATERIALS AND METHODS

### TOXOID PREPARATIONS

#### Formalin

5 ml. of the various formalin solutions were added to each of a number of vials containing 10 mg. of highly purified freeze-dried Staphylococcal enterotoxin, Lot No. 14-31. After maintenance under the designated experimental conditions, the vials were stored at  $-20^{\circ}\text{C}$ .

1. 0.2% formalin at  $37^{\circ}\text{C}$ . for 1 week
2. " " " " " 2 weeks
3. " " " " " 3 weeks
4. 0.2% formalin at  $5^{\circ}\text{C}$ . for 6 weeks
5. 0.8% formalin at  $37^{\circ}\text{C}$ . for 1 week
6. " " " " " 2 weeks
7. " " " " " 3 weeks
8. 0.8% formalin at  $5^{\circ}\text{C}$ . for 6 weeks
9. 0.2% formalin 0.15% glycine at  $25^{\circ}\text{C}$ . for 1 week
10. " " " " " " 2 weeks
11. " " " " " " 3 weeks

#### Hexamethylenetetramine

12. 0.3% hexamethylenetetramine at  $37^{\circ}\text{C}$ . for 3 weeks
13. 1.0% " " " " " "

#### Beta-Propiolactone

14. 5 ml. of a chilled 0.2% solution of beta-propiolactone in isotonic disodium hydrogen phosphate was added to each of a number of vials containing 10 mg. of toxin, and held at  $37^{\circ}\text{C}$ . for 2 hours. The reaction was allowed to proceed overnight at room temperature, and then stored at  $-20^{\circ}\text{C}$ .

15. Same as above except for change to 1.0% beta-propiolactone.

#### Diethyl Pyrocarbonate

16. 5 ml. of a chilled 0.2% solution of diethyl pyrocarbonate in ethanol-water (1:1) was added to each of a number of vials containing 10 mg. of toxin, and stored for 1 week at 5°C. The vials were then held overnight at room temperature, and then stored at -20°C.

17. Same as above except for change to 1.0% diethyl pyrocarbonate.

#### Ethylene Oxide

18. 5 ml. of a chilled 0.2% solution of ethylene oxide was added to each of a number of vials containing 10 mg. of toxin. The vials were held at 5°C. for 2 hours, maintained overnight at room temperature, and stored at -20°C.

19. Same as above except for change to 1.0% ethylene oxide.

20. A number of vials of toxin, 10 mg./vial, were placed in a vacuum desiccator along with 5 ml. of liquefied ethylene oxide contained in a small Erlenmyer flask. The vacuum desiccator was partially evacuated to allow for the expansion of ethylene oxide vapor. After 24 hour exposure in the ethylene oxide atmosphere, the trace amounts of adsorbed ethylene oxide were removed by exhausting the vacuum desiccator for several hours. The vials were stored at -20°C.

#### Ethyleneimine

21. A number of vials of toxin, 10 mg./vial, were placed in a vacuum desiccator along with 5 ml. of ethyleneimine contained in a small Erlenmyer flask. The vacuum desiccator was partially evacuated to allow for the expansion of ethyleneimine vapor. After 24 hours exposure in the ethyleneimine atmosphere, the vacuum desiccator was exhausted for 24 hours. The vials were stored at -20°C.

#### Methyl Bromide

22. A number of vials of toxin, 10 mg./vial, were placed in a vacuum desiccator along with 5 ml. of liquefied methyl bromide contained in a small Erlenmyer flask. The vacuum desiccator was partially evacuated to allow for the expansion of methyl bromide vapor. After 24 hours exposure in the methyl bromide atmosphere, the trace amounts of adsorbed methyl bromide were removed by exhausting for several hours.

#### Propylene Oxide

23. Procedure identical to that described for ethylene oxide and methyl bromide.

#### Methanol Esterification

24. Two ml. of absolute methanol saturated with dry hydrogen chloride was added to each of a number of vials containing 10 mg. of toxin. After standing for 3 days at room temperature, the vials were taken to dryness in vacuo. Two ml. of sterile distilled water was then added to each vial, the material freeze-dried, and stored at  $-20^{\circ}\text{C}$ .

#### Sodium Gluconate

25. 5 ml. of an aqueous 1.0% solution of sodium gluconate was added to each of a number of vials containing 10 mg. of toxin. After holding for 4 weeks at  $37^{\circ}\text{C}$ ., the vials were stored at  $-20^{\circ}\text{C}$ .

#### Ultraviolet Light Irradiation

26. 250 mg. of toxin was dissolved in water and transferred in a final volume of 125 ml. to a stoppered quartz Erlenmeyer flask fitted with a magnetic stirrer, and cooled by running water. The stirred solution was exposed to the rays from an ultraviolet light lamp, short wave (2537 Angstrom units), fixed at a distance of 20 cm., for 1 hour. Two ml. aliquots were transferred to a number of sterile vials, and stored at  $-20^{\circ}\text{C}$ .

27. Same as above except for exposure time of 5 hours.

#### Photodynamic Action of Methylene Blue

28. 250 mg. of toxin was dissolved in an aqueous solution of methylene blue (1:10,000), and transferred in a final volume of 125 ml. to a stoppered Erlenmeyer flask fitted with a magnetic stirrer, and cooled by running water. The stirred solution was exposed to the light from a 100 Watt incandescent bulb fixed at a distance of 20 cm. for 1 hour. Two ml. aliquots were transferred to a number of sterile vials, and stored at  $-20^{\circ}\text{C}$ .

29. Same as above except for exposure time of 8 hours.

#### Acetic Anhydride

30. To 250 mg. of toxin dissolved in 5 ml. of saturated sodium acetate solution and cooled in an ice bath, was added with stirring 0.3 ml. of acetic anhydride. After stirring for an hour, the material was carefully transferred to a small cellophane bag, and dialyzed against distilled water in the refrigerator for 48 hours with frequent changes of water. Two ml. portions of the opalescent solution were transferred to each of a number of sterile vials, 4 mg./vial, and stored at  $-20^{\circ}\text{C}$ .

### Adjuvant

A light mineral oil (Drakeol 6VR) and a mannide monoleate (Arlacel A) were mixed in a 9:1 ratio, and sterilized by Seitz filtration.

### Emulsification

To each vial containing 1 ml. of toxoid solution, 2 mg./ml. was added an equal volume of the mineral oil - Arlacel A solution. Emulsions of the vaccines were prepared directly in the vials by forcing the contents back and forth through a 1 ml. glass tuberculin syringe fitted with a 20 gauge needle. The process was continued until a drop of emulsion expelled through the hypodermic needle into a beaker of cold water retained its shape.

### Immunization Schedule

#### Range Finding Studies

Pairs of rhesus monkeys were immunized with each of the thirty different toxoid preparations. The immunization schedule consisted of three subcutaneous injections spaced at five week intervals. A total dose of 0.10 mg. was given in the first injection. The second and third injections each consisted of a total dose of 0.25 mg. of toxoid.

The thirty pairs of monkeys, previously immunized with the thirty different enterotoxoid samples, were challenged intravenously intravenously three weeks after the third and final immunization injections with untreated Staphylococcal enterotoxin dissolved in 0.02 M phosphate buffer, pH 7.4. One monkey of each pair was challenged at 10 /kg., and the other at 1000 /kg.

#### Extended Evaluation Studies

Twelve monkeys were immunized, in the manner already described, with each of the ten selected toxoid samples. Four monkeys from each set are to be challenged intravenously with untreated Staphylococcal enterotoxin six months after the third and final immunization injections. At the same time, a second set of four monkeys from each group is to be given a booster shot of 0.25 mg. of toxoid. These monkeys, along with the third set of four monkeys from each group, are to be challenged intravenously with untreated Staphylococcal enterotoxin one year after the initial immunization period.

### Toxicity Determinations

After each injection of Staphylococcal enterotoxin or toxoid, absence or presence of illness (emesis, diarrhea) was noted through a five hour observation period. Death or delayed death with marked weight loss was interpreted as a direct toxic manifestation of the injected material.



### Serologic Tests

Antibody titers were determined by hemagglutination and gel-diffusion tests as performed at Fort Detrick.

The lowest dilution of sera in the hemagglutination test was arbitrarily set at 1:10,000. Titers are expressed as the reciprocal of the highest serum dilution showing gross hemagglutination.

In the gel-diffusion assay, a preliminary qualitative test was run in single tubes with sera diluted 1:10 overlaid with Staphylococcal enterotoxin 100  $\mu$ /ml. Samples which gave sharp, demonstrable zones after twenty four hours were assayed in the usual eight tube quantitative test. Titers are expressed as reciprocals of the number.

### RESULTS

#### Range Finding Studies

Some toxicity (emesis, diarrhea) was observed following the first subcutaneous immunization injection of the Staphylococcal enterotoxinoid samples. There were no deaths. No toxic reactions followed the second and third immunization injections.

Antibody titers were generally low or negative after the first immunization injections. Many of the antibody titers rose sharply after the second and third subcutaneous immunization injections.

Out of thirty monkeys (each immunized with a different Staphylococcal enterotoxinoid) challenged intravenously with untreated Staphylococcal enterotoxin at 10  $\mu$ /kg., there was an illness response in only two monkeys. There were no deaths.

Out of twenty nine monkeys challenged intravenously with untreated Staphylococcal enterotoxin at 1000  $\mu$ /kg., there were two deaths and fifteen illness responses. Twelve monkeys had no reactions.

The correlation between antibody titers and protection against intravenous challenge in these range finding studies was suggestive but not clear cut.

### Extended Evaluation Studies

The following ten preparations were selected from the range finding studies for more extended comparisons of toxicities, antibody response, and degree and duration of immunity to intravenous challenge with untreated Staphylococcal enterotoxin:

| <u>Sample No.</u> | <u>Treatment</u>                                   |
|-------------------|--|
| 2                 | 0.2% formalin at 37°C. for 2 weeks                 |
| 10                | 0.2% formalin + 0.15% glycine at 25°C. for 2 weeks |
| 12                | 0.3% hexamethylenetetramine at 37°C. for 3 weeks   |
| 14                | 0.2% beta-propiolactone at 37°C. for 2 hours       |
| 16                | 0.2% diethyl pyrocarbonate at 5°C. for 1 week      |
| 18                | 0.2% ethylene oxide at 5°C. for 2 hours            |
| 22                | Exposure to methyl bromide vapor for 24 hours      |
| 25                | 1.0% sodium gluconate at 37°C. for 4 weeks         |
| 26                | Exposure to ultraviolet light for 1 hour           |
| 28                | Photodynamic action of methylene blue for 1 hour   |

Sets of twelve monkeys were immunized with each of the ten toxoid samples. The following table shows the toxicity responses to the first subcutaneous immunization injection of 0.10 mg. of toxoid with adjuvant:

| <u>Sample No.</u> | <u>Illness</u> | <u>Death</u> | <u>No Reaction</u> |
|-------------------|----------------|--------------|--------------------|
| 2                 | 6              | 0            | 6                  |
| 10                | 0              | 0            | 12                 |
| 12                | 8              | 0            | 4                  |
| 14                | 10             | 1            | 1                  |
| 16                | 8              | 2            | 2                  |
| 18                | 9              | 1            | 2                  |
| 22                | 6              | 0            | 6                  |
| 25                | 11             | 0            | 1                  |
| 26                | 5              | 0            | 7                  |
| 28                | 9              | 1            | 2                  |

Sample #10 is the most impressive preparation to date. In a separate experiment, three monkeys were injected subcutaneously with 0.25 mg. of toxoid. There were no reactions. In addition to being the least toxic of the preparations tested, Sample #10 produced the highest antibody titers as measured by hemagglutination and gel-diffusion.

### SUMMARY AND CONCLUSIONS

The range finding studies concerned with the preliminary evaluation of thirty Staphylococcal enterotoxoid samples have been completed.

Thirty pairs of rhesus monkeys, previously immunized with thirty different toxoid preparations, were challenged intravenously with untreated Staphylococcal enterotoxin three weeks after the third and final subcutaneous immunization injection.

Out of thirty monkeys (each immunized with a different Staphylococcal enterotoxoid) challenged intravenously with untreated enterotoxin at 10 v/mg., there was an illness response in only two monkeys. There were no deaths.

Out of twenty nine monkeys challenged intravenously with enterotoxin at 1000 v/kg., there were two deaths and fifteen illness responses. There were no reactions in twelve monkeys.

On the basis of toxicity, antibody titers, and intravenous protection results, ten Staphylococcal toxoid preparations were selected for more extended evaluation.

Sample #10 (0.2% formalin+0.15% glycine at 25°C. for 2 weeks) is the most impressive of the toxoid preparations on the basis of toxicity and antigenic response. The final evaluation, scheduled for May 30, 1965, will depend on the degree and duration of immunity to intravenous challenge with untreated Staphylococcal enterotoxin.

The attached summary sheets contain all the tabulated data available to date. Additional entries will be made as the experimental studies are extended.

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Sample #2

0.2% formalin at 37°C. for 2 weeks

Date

| <p>Monkey #49</p> <p>Immunization<br/>Dose, mg. (SQ)<br/>Reaction<br/>Antibody Titer<br/>Hemagglutination<br/>Gel-Diffusion<br/>Intravenous Challenge<br/>Dose (mcg./kg.)<br/>Reaction</p> | 9/24/63      | 10/21/63       | 10/28/63     | 11/25/63       | 12/3/63      | 12/17/63         | 12/23/63     |
|--|--------------|----------------|--------------|----------------|--------------|------------------|--------------|
|  | 0.10<br>N.R. | 160,000<br>204 | 0.25<br>N.R. | 320,000<br>100 | 0.25<br>N.R. | 1,280,000<br>400 | 10.0<br>N.R. |
| <p>Monkey #50</p> <p>Immunization<br/>Dose, mg. (SQ)<br/>Reaction<br/>Antibody Titer<br/>Hemagglutination<br/>Gel-Diffusion<br/>Intravenous Challenge<br/>Dose (mcg./kg.)<br/>Reaction</p> | 9/24/63      | 10/21/63       | 10/28/63     | 11/25/63       | 12/3/63      | 12/17/63         | 12/23/63     |
|  | 0.25<br>N.R. | 320,000<br>190 | 0.25<br>N.R. | 320,000<br>240 | 0.25<br>N.R. | 1,280,000<br>205 | 1000<br>I    |

Sample #3

0.2% formalin at 37° C. for 3 weeks

Date

|  | 10/1/63      | 10/28/63            | 11/4/63      | 12/3/63           | 12/9/63      | 12/23/63           | 12/31/63     |
|--|--------------|---------------------|--------------|-------------------|--------------|--------------------|--------------|
| <u>Monkey #52</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>N.R. | 160,000<br>Positive | 0.25<br>N.R. | 2,560,000<br>159  | 0.25<br>N.R. | 2,560,000<br>390   | 10.0<br>N.R. |
| <u>Monkey #56</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.25<br>I    | <10,000<br>No Zone  | 0.25<br>N.R. | 20,000<br>No Zone | 0.25<br>N.R. | <10,000<br>No Zone | 1000<br>I    |



Sample #4

0.2% formalin at 5°C. for 6 weeks

Date

|   | Date      |                      |          |              |               |              |                  |           |  |  |
|---|-----------|----------------------|----------|--------------|---------------|--------------|------------------|-----------|--|--|
|   | 10/21/63  | 11/18/63             | 11/21/63 | 11/25/63     | 12/23/63      | 12/30/63     | 1/10/64          | 1/24/64   |  |  |
| <b>Monkey #14</b><br>Immunization<br>Dose, mg. (S <sub>1</sub> )<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I | 160,000<br>Positive  |          | 0.25<br>N.R. | 320,000<br>50 | 0.25<br>N.R. | 1,280,000<br>347 | 1000<br>X |  |  |
| <b>Monkey #61</b><br>Immunization<br>Dose, mg. (S <sub>1</sub> )<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion   | 0.25<br>I | < 10,000<br>Positive | X        |              |               |              |                  |           |  |  |

Sample #5

Sample #5

0.8% formalin at 37°C. for 1 week

Date

|  | 9/18/63      | 10/15/63            | 10/23/63     | 11/25/63           | 12/9/63      | 12/17/63         | 12/23/63     |
|--|--------------|---------------------|--------------|--------------------|--------------|------------------|--------------|
| <u>Monkey #43</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I    | <10,000<br>Positive | 0.25<br>N.R. | 40,000<br>Positive | 0.25<br>N.R. | 1,280,000<br>400 | 10.0<br>N.R. |
| <u>Monkey #44</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.25<br>N.R. | <10,000<br>No Zone  | 0.25<br>N.R. | 20,000<br>Positive | 0.25<br>N.R. | 320,000<br>160   | 1000<br>N.R. |

Sample #6

0.8% formalin at 37°C. for 2 weeks

Date

|  | 9/24/63      | 10/21/63            | 10/28/63     | 11/25/63           | 12/8/63      | 12/17/63       | 12/23/63     |
|--|--------------|---------------------|--------------|--------------------|--------------|----------------|--------------|
| <b>Monkey #51</b><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>N.R. | 10,000<br>No Zone   | 0.25<br>N.R. | 80,000<br>129      | 0.25<br>N.R. | 640,000<br>200 | 10.0<br>N.R. |
| <b>Monkey #52</b><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.25<br>N.R. | < 10,000<br>No Zone | 0.25<br>N.R. | 40,000<br>Positive | 0.25<br>N.R. | 640,000<br>160 | 1000<br>N.R. |

Sample #7

0.8% formalin at 37°C. for 3 weeks

Date

|  | 10/1/63      | 10/28/63            | 11/4/63      | 12/3/63             | 12/9/63      | 12/23/63          | 12/31/63     |
|--|--------------|---------------------|--------------|---------------------|--------------|-------------------|--------------|
| <b>Monkey #51</b><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>N.R. | < 10,000<br>No Zone | 0.25<br>N.R. | < 10,000<br>No Zone | 0.25<br>N.R. | 20,000<br>No Zone | 10.0<br>N.R. |
| <b>Monkey #58</b><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.25<br>I    | < 10,000<br>No Zone | 0.25<br>N.R. | 80,000<br>Positive  | 0.25<br>N.R. | 80,000<br>No Zone | 1000<br>I    |

Sample #8

0.8% formalin at 5°C. for 6 weeks

Date

|   | <u>Date</u> |                     |              |                    |              |                      |
|---|-------------|---------------------|--------------|--------------------|--------------|----------------------|
|   | 10/21/63    | 11/18/63            | 11/25/63     | 12/23/63           | 12/30/63     | 1/10/64              |
| <p><u>Monkey #62</u></p> <p>Immunization<br/>Dose, mg. (S.)<br/>Reaction<br/>Antibody Titer<br/>Hemagglutination<br/>Gel-Diffusion<br/>Intravenous Challenge<br/>Dose (mcg./kg.)<br/>Reaction</p> | 0.10<br>I   | <10,000<br>No Zone  | 0.25<br>N.R. | 20,000<br>No Zone  | 0.25<br>N.R. | 2,550,000<br>No Zone |
|   |             |                     |              |                    |              | 1000<br>I            |
| <p><u>Monkey #53</u></p> <p>Immunization<br/>Dose, mg. (S.)<br/>Reaction<br/>Antibody Titer<br/>Hemagglutination<br/>Gel-Diffusion<br/>Intravenous Challenge<br/>Dose (mcg./kg.)<br/>Reaction</p> | 0.25<br>I   | <10,000<br>Positive | 0.25<br>N.R. | <10,000<br>No Zone | 0.25<br>N.R. | 90,000<br>No Zone    |
|   |             |                     |              |                    |              | 10.0<br>N.R.         |

Sample #9

0.2% formalin + 0.15% glycine at 25°C. for 1 week

Date

| <p> <u>Monkey #41</u><br/>           Immunization<br/>           Dose, mg. (SQ)<br/>           Reaction<br/>           Antibody Titer<br/>           Hemagglutination<br/>           Gel-Diffusion<br/>           Intravenous Challenge<br/>           Dose (mcg./kg.)<br/>           Reaction         </p> | 9/18/63      | 10/15/63           | 10/23/63     | 11/25/63            | 12/9/63      | 12/17/63         | 12/23/63     |
|---|--------------|--------------------|--------------|---------------------|--------------|------------------|--------------|
|   | 0.10<br>N.R. | 160,000<br>No Zone | 0.25<br>N.R. | 320,000<br>Positive | 0.25<br>N.R. | 1,256,000<br>380 | 10.0<br>N.R. |
| <p> <u>Monkey #42</u><br/>           Immunization<br/>           Dose, mg. (SQ)<br/>           Reaction<br/>           Antibody Titer<br/>           Hemagglutination<br/>           Gel-Diffusion<br/>           Intravenous Challenge<br/>           Dose (mcg./kg.)<br/>           Reaction         </p> | 0.25<br>I    | <10,000<br>No Zone | 0.25<br>N.R. | 160,000<br>Positive | 0.25<br>N.R. | 160,000<br>50    | 1000<br>I    |
|   |              |                    |              |                     |              |                  |              |

Sample #10

0.2% formalin + 0.15% glycine at 25°C. for 2 weeks

Date

|  | 9/24/63   | 10/21/63          | 10/28/63     | 11/25/63           | 12/3/63      | 12/17/63       | 12/23/63     |
|--|-----------|-------------------|--------------|--------------------|--------------|----------------|--------------|
| <u>Monkey #53</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I | 10,000<br>No Zone | 0.25<br>N.R. | 160,000<br>209     | 0.25<br>N.R. | 640,000<br>400 | 10.0<br>N.R. |
| <u>Monkey #54</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.25<br>I | 10,000<br>No Zone | 0.25<br>N.R. | 40,000<br>Positive | 0.25<br>N.R. | 320,000<br>50  | 1000<br>I    |

Sample #11

0.2% formalin + 0.15% glycine at 25°C. for 3 weeks

Date

|  | 10/1/63   | 10/28/63           | 11/4/63      | 12/3/63            | 12/9/63      | 12/23/63           | 12/31/63     |
|--|-----------|--------------------|--------------|--------------------|--------------|--------------------|--------------|
| <b>Monkey #59</b><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I | <10,000<br>No Zone | 0.25<br>N.R. | 80,000<br>Positive | 0.25<br>N.R. | 320,000<br>No Zone | 10.0<br>N.R. |
| <b>Monkey #60</b><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.25<br>I | <10,000<br>No Zone | 0.25<br>N.R. | 20,000<br>No Zone  | 0.25<br>N.R. | 80,000<br>No Zone  | 1000<br>I    |



Sample #12

0.3% hexamethylenetetramine at 37°C. for 3 weeks

Date

|                             | 9/11/63 | 10/7/63 | 10/16/63 | 11/8/63  | 11/19/63 | 12/2/63 | 12/10/63 |
|-----------------------------|---------|---------|----------|----------|----------|---------|----------|
| <u>Monkey #16</u>           |         |         |          |          |          |         |          |
| Immunization                |         |         |          |          |          |         |          |
| Dose, mg. (S <sub>1</sub> ) | 0.10    |         | 0.25     |          | 0.25     |         |          |
| Reaction                    | N.R.    |         | N.R.     |          | N.R.     |         |          |
| Antibody Titer              |         |         |          |          |          |         |          |
| Hemagglutination            |         | 160,000 |          | 160,000  |          | 160,000 |          |
| Gel-Diffusion               |         | No Zone |          | Positive |          | 87      |          |
| Intravenous Challenge       |         |         |          |          |          |         |          |
| Dose (mcg./kg.)             |         |         |          |          |          |         | 10.0     |
| Reaction                    |         |         |          |          |          |         | N.R.     |
| <u>Monkey #17</u>           |         |         |          |          |          |         |          |
| Immunization                |         |         |          |          |          |         |          |
| Dose, mg. (S <sub>2</sub> ) | 0.25    |         | 0.25     |          | 0.25     |         |          |
| Reaction                    | I       |         | N.R.     |          | N.R.     |         |          |
| Antibody Titer              |         |         |          |          |          |         |          |
| Hemagglutination            |         | 80,000  |          | 320,000  |          | 320,000 |          |
| Gel-Diffusion               |         | No Zone |          | 100      |          | 230     |          |
| Intravenous Challenge       |         |         |          |          |          |         |          |
| Dose (mcg./kg.)             |         |         |          |          |          |         | 1000     |
| Reaction                    |         |         |          |          |          |         | N.R.     |

Sample #13

1.0% hexamethylenetetramine at 37°C. for 3 weeks

Date

|                             | 9/11/63 | 10/7/63 | 10/16/63 | 11/8/63  | 11/19/63 | 12/2/63  | 12/10/63 |
|-----------------------------|---------|---------|----------|----------|----------|----------|----------|
| <u>Monkey #13</u>           |         |         |          |          |          |          |          |
| Immunization                |         |         |          |          |          |          |          |
| Dose, mg. (S <sub>2</sub> ) | 0.10    |         | 0.25     | 320,000  | 0.25     | 80,000   | 10.0     |
| Reaction                    | N.R.    |         | N.R.     | 305      | N.R.     | Positive | N.R.     |
| Antibody Titer              |         |         |          |          |          |          |          |
| Hemagglutination            |         | 80,000  |          |          |          |          |          |
| Gel-Diffusion               |         | No Zone |          |          |          |          |          |
| Intravenous Challenge       |         |         |          |          |          |          |          |
| Dose (mcg./kg.)             |         |         |          |          |          |          |          |
| Reaction                    |         |         |          |          |          |          |          |
| <u>Monkey #15</u>           |         |         |          |          |          |          |          |
| Immunization                |         |         |          |          |          |          |          |
| Dose, mg. (S <sub>4</sub> ) | 0.25    |         | 0.25     | 160,000  | 0.25     | 80,000   | 1000     |
| Reaction                    | I       |         | N.R.     | Positive | F.R.     | Positive | N.R.     |
| Antibody Titer              |         |         |          |          |          |          |          |
| Hemagglutination            |         | 320,000 |          |          |          |          |          |
| Gel-Diffusion               |         | No Zone |          |          |          |          |          |
| Intravenous Challenge       |         |         |          |          |          |          |          |
| Dose (mcg./kg.)             |         |         |          |          |          |          |          |
| Reaction                    |         |         |          |          |          |          |          |

Sample #14

0.2% beta-propiolactone at 37°C. for 2 hours

Date

|                       | 9/10/63 | 10/7/63  | 10/14/63 | 11/8/63  | 11/18/63 | 12/2/63  | 12/10/63 |
|-----------------------|---------|----------|----------|----------|----------|----------|----------|
| <u>Monkey #3</u>      |         |          |          |          |          |          |          |
| Immunization          |         |          |          |          |          |          |          |
| Dose, mg. (SQ)        | 0.10    |          | 0.25     |          | 0.25     |          |          |
| Reaction              | I       |          | N.R.     |          | N.R.     |          |          |
| Antibody Titer        |         |          |          |          |          |          |          |
| Hemagglutination      |         | 20,000   |          | 320,000  |          | 800,000  |          |
| Gel-Diffusion         |         | No Zone  |          | Positive |          | 174      |          |
| Intravenous Challenge |         |          |          |          |          |          |          |
| Dose (mcg./kg.)       |         |          |          |          |          |          | 10.0     |
| Reaction              |         |          |          |          |          |          | N.R.     |
| <u>Monkey #4</u>      |         |          |          |          |          |          |          |
| Immunization          |         |          |          |          |          |          |          |
| Dose, mg. (SQ)        | 0.25    |          | 0.25     |          | 0.25     |          |          |
| Reaction              | I       |          | N.R.     |          | N.R.     |          |          |
| Antibody Titer        |         |          |          |          |          |          |          |
| Hemagglutination      |         | < 10,000 |          | 80,000   |          | 160,000  |          |
| Gel-Diffusion         |         | No Zone  |          | No Zone  |          | Positive |          |
| Intravenous Challenge |         |          |          |          |          |          |          |
| Dose (mcg./kg.)       |         |          |          |          |          |          | 1000     |
| Reaction              |         |          |          |          |          |          | I        |

Sample #15

1.0% beta-propiolactone at 37°C. for 2 hours

Date

|  | 9/10/63   | 10/7/63            | 10/14/63     | 11/8/63             | 11/18/63     | 12/2/63            | 12/10/63     |
|--|-----------|--------------------|--------------|---------------------|--------------|--------------------|--------------|
| <u>Monkey #2</u><br>Immunization<br>Dose, mg. (S)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I | <10,000<br>No Zone | 0.25<br>N.R. | 80,000<br>Positive  | 0.25<br>N.R. | 80,000<br>Positive | 10.0<br>N.R. |
| <u>Monkey #5</u><br>Immunization<br>Dose, mg. (S)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.25<br>I | <10,000<br>No Zone | 0.25<br>N.R. | 160,000<br>Positive | 0.25<br>N.R. | 80,000<br>Positive | 1000<br>N.R. |

Sample #16

0.2% diethyl pyrocarbonate at 5°C. for 1 week

Date

| <p><u>Monkey #11</u></p> <p>Immunization<br/>Dose, mg. (SQ)<br/>Reaction<br/>Antibody Titer<br/>Hemagglutination<br/>Gel-Diffusion<br/>Intravenous Challenge<br/>Dose (mcg./kg.)</p>              | 9/10/63   | 10/7/63           | 10/14/63     | 11/8/63            | 11/18/63     | 12/2/63             | 12/10/63     |
|---|-----------|-------------------|--------------|--------------------|--------------|---------------------|--------------|
|   | 0.10<br>I | 80,000<br>213     | 0.25<br>N.R. | 80,000<br>Positive | 0.25<br>N.R. | 20,000<br>Positive  | 10.0<br>N.R. |
| <p><u>Monkey #12</u></p> <p>Immunization<br/>Dose, mg. (SQ)<br/>Reaction<br/>Antibody Titer<br/>Hemagglutination<br/>Gel-Diffusion<br/>Intravenous Challenge<br/>Dose (mcg./kg.)<br/>Reaction</p> | 9/10/63   | 10/7/63           | 10/14/63     | 11/8/63            | 11/18/63     | 12/2/63             | 12/10/63     |
|   | 0.25<br>I | 20,000<br>No Zone | 0.25<br>N.R. | 80,000<br>No Zone  | 0.25<br>N.R. | 160,000<br>Positive | 1000<br>N.R. |

Sample #17

1.0% diethyl pyrocarbonate at 5°C. for 1 week

Date

|  | 9/18/63      | 10/15/63          | 10/23/63     | 11/25/63          | 12/9/63      | 12/17/63          | 12/23/63  |
|--|--------------|-------------------|--------------|-------------------|--------------|-------------------|-----------|
| <b>Monkey #45</b><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>N.R. | 10,000<br>No Zone | 0.25<br>N.R. | 10,000<br>No Zone | 0.25<br>N.R. | 10,000<br>No Zone | 10.0<br>I |
|  | 0.25<br>N.R. | 10,000<br>No Zone | 0.25<br>N.R. | 10,000<br>No Zone | 0.25<br>N.R. | 20,000<br>No Zone | 1000<br>I |
| <b>Monkey #46</b><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>N.R. | 10,000<br>No Zone | 0.25<br>N.R. | 10,000<br>No Zone | 0.25<br>N.R. | 10,000<br>No Zone | 10.0<br>I |
|  | 0.25<br>N.R. | 10,000<br>No Zone | 0.25<br>N.R. | 10,000<br>No Zone | 0.25<br>N.R. | 20,000<br>No Zone | 1000<br>I |

Sample #18

0.2% ethylene oxide at 5°C. for 2 hours

Date

|                             | 9/10/63 | 10/7/63 | 10/14/63 | 11/8/63  | 11/18/63 | 12/2/63  | 12/10/63 |
|-----------------------------|---------|---------|----------|----------|----------|----------|----------|
| <u>Monkey #9</u>            |         |         |          |          |          |          |          |
| Immunization                |         |         |          |          |          |          |          |
| Dose, mg. (S <sub>4</sub> ) | 0.10    |         | 0.25     | 160,000  | 0.25     | 320,000  | 10.0     |
| Reaction                    | I       |         | N.R.     | 205      | N.R.     | 345      | N.R.     |
| Antibody Titer              |         | 20,000  |          |          |          |          |          |
| Hemagglutination            |         | 100     |          |          |          |          |          |
| Gel-Diffusion               |         |         |          |          |          |          |          |
| Intravenous Challenge       |         |         |          |          |          |          |          |
| Dose (mcg./kg.)             |         |         |          |          |          |          |          |
| Reaction                    |         |         |          |          |          |          |          |
| <u>Monkey #10</u>           |         |         |          |          |          |          |          |
| Immunization                |         |         |          |          |          |          |          |
| Dose, mg. (S <sub>4</sub> ) | 0.25    |         | 0.25     | 80,000   | 0.25     | 80,000   | 1000     |
| Reaction                    | I       |         | N.R.     | Positive | N.R.     | Positive | N.R.     |
| Antibody Titer              |         | 10,000  |          |          |          |          |          |
| Hemagglutination            |         | No Zone |          |          |          |          |          |
| Gel-Diffusion               |         |         |          |          |          |          |          |
| Intravenous Challenge       |         |         |          |          |          |          |          |
| Dose (mcg./kg.)             |         |         |          |          |          |          |          |
| Reaction                    |         |         |          |          |          |          |          |

Sample #19

1.0% ethylene oxide at 5°C. for 2 hours

Date

|  | 9/11/63      | 10/8/63            | 10/16/63     | 11/8/63             | 11/19/63     | 12/2/63           | 12/10/63     |
|--|--------------|--------------------|--------------|---------------------|--------------|-------------------|--------------|
| <u>Monkey #22</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I    | <10,000<br>No Zone | 0.25<br>N.R. | 160,000<br>Positive | 0.25<br>N.R. | 160,000<br>175    | 10.0<br>N.R. |
| <u>Monkey #23</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.25<br>N.R. | <10,000<br>No Zone | 0.25<br>N.R. | <10,000<br>No Zone  | 0.25<br>N.R. | 20,000<br>No Zone | 1000<br>I    |



Sample #20

Exposure to ethylene oxide vapor for 24 hours

Date

|  | 9/11/63      | 10/8/63           | 10/16/63     | 11/8/63           | 11/19/63     | 12/2/63           | 12/10/63     |  |
|--|--------------|-------------------|--------------|-------------------|--------------|-------------------|--------------|--|
| <u>Monkey #20</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>N.R. | 10,000<br>No Zone | 0.50<br>I    | 10,000<br>No Zone | 0.50<br>N.R. | 20,000<br>No Zone | 10.0<br>N.R. |  |
|  | 0.25<br>N.R. | 20,000<br>No Zone | 0.50<br>N.R. | 40,000<br>No Zone | 0.50<br>N.R. | 20,000<br>No Zone | 1000<br>I    |  |
| <u>Monkey #21</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction |              |                   |              |                   |              |                   |              |  |
|  |              |                   |              |                   |              |                   |              |  |

Sample #21

Exposure to ethyleneimine vapor for 24 hours

Date

|  | 9/12/63      | 10/8/63             | 10/17/63     | 11/11/63            | 11/20/63     | 12/2/63             | 12/10/63     |
|--|--------------|---------------------|--------------|---------------------|--------------|---------------------|--------------|
|  | 10/12/63     | 10/17/63            | 11/11/63     | 11/20/63            | 12/2/63      | 12/10/63            | 12/10/63     |
| <u>Monkey #34</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>N.R. | 10,000<br>No Zone   | 0.50<br>N.R. | < 10,000<br>No Zone | 0.25<br>N.R. | 20,000<br>No Zone   | 10.0<br>N.R. |
| <u>Monkey #35</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.25<br>N.R. | < 10,000<br>No Zone | 0.50<br>N.R. | < 10,000<br>No Zone | 0.25<br>N.R. | < 10,000<br>No Zone | 1000<br>X    |

Exposure to methyl bromide vapor for 24 hours

Sample #23

Sample #23

Exposure to propylene oxide vapor for 24 hours

Date

|  | 9/11/63   | 10/8/63           | 10/16/63     | 11/8/63            | 11/19/63     | 12/2/63             | 12/10/63     |
|--|-----------|-------------------|--------------|--------------------|--------------|---------------------|--------------|
| <b>Monkey #24</b><br>Immunization<br>Dose, mg. (Sq)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I | 10,000<br>No Zone | 0.25<br>N.R. | 10,000<br>No Zone  | 0.25<br>N.R. | 20,000<br>No Zone   | 10.0<br>N.R. |
|  | 0.25<br>I | 80,000<br>No Zone | 0.25<br>N.R. | 80,000<br>Positive | 0.25<br>N.R. | 320,000<br>Positive | 1000<br>I    |
| <b>Monkey #25</b><br>Immunization<br>Dose, mg. (Sq)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction |           |                   |              |                    |              |                     |              |
|  |           |                   |              |                    |              |                     |              |

Sample #24

Methanol esterification

Date

|  | <u>Date</u>  |                     |              |                     |              |                     |
|--|--------------|---------------------|--------------|---------------------|--------------|---------------------|
|  | 9/11/63      | 10/8/63             | 10/16/63     | 11/8/63             | 11/19/63     | 12/2/63             |
| <b>Monkey #18</b><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>N.R. | < 10,000<br>No Zone | 0.50<br>N.R. | < 10,000<br>No Zone | 0.50<br>N.R. | < 10,000<br>No Zone |
|  |              |                     |              |                     |              | 10.0<br>I           |
| <b>Monkey #19</b><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.25<br>N.R. | < 10,000<br>No Zone | 0.50<br>N.R. | < 10,000<br>No Zone | 0.50<br>N.R. | < 10,000<br>No Zone |
|  |              |                     |              |                     |              | 1.0<br>N.R.         |

Sample #26

Exposure to ultraviolet light for 1 hour

Date

| <p><u>Monkey #36</u></p> <p>Immunization<br/>Dose, mg. (sq)<br/>Reaction<br/>Antibody Titer<br/>Hemagglutination<br/>Gel-Diffusion<br/>Intravenous Challenge<br/>Dose (mcg./kg.)<br/>Reaction</p> | 9/12/63      | 10/8/63           | 10/17/63     | 11/11/63           | 11/20/63     | 12/3/63             | 12/10/63     |
|---|--------------|-------------------|--------------|--------------------|--------------|---------------------|--------------|
|   | 0.10<br>I    | 20,000<br>No Zone | 0.25<br>H.R. | 80,000<br>Positive | 0.25<br>H.R. | 320,000<br>Positive | 10.0<br>H.R. |
| <p><u>Monkey #37</u></p> <p>Immunization<br/>Dose, mg. (sq)<br/>Reaction<br/>Antibody Titer<br/>Hemagglutination<br/>Gel-Diffusion<br/>Intravenous Challenge<br/>Dose (mcg./kg.)<br/>Reaction</p> | 0.25<br>H.R. | 40,000<br>No Zone | 0.25<br>H.R. | 10,000<br>Positive | 0.25<br>H.R. | 640,000<br>No Zone  | 1000<br>I    |
|   |              |                   |              |                    |              |                     |              |

Sample #26

Exposure to ultraviolet light for 1 hour

Date

| <p><u>Monkey #36</u></p> <p>Immunization<br/>Dose, mg. (SQ)<br/>Reaction<br/>Antibody Titer<br/>Hemagglutination<br/>Gel-Diffusion<br/>Intravenous Challenge<br/>Dose (mcg./kg.)<br/>Reaction</p> | 9/12/63      | 10/8/63           | 10/17/63     | 11/11/63           | 11/20/63     | 12/3/63             | 12/10/63     |  |
|---|--------------|-------------------|--------------|--------------------|--------------|---------------------|--------------|--|
|   | 0.10<br>I    | 20,000<br>No Zone | 0.25<br>N.R. | 80,000<br>Positive | 0.25<br>N.R. | 320,000<br>Positive | 10.0<br>N.R. |  |
| <p><u>Monkey #37</u></p> <p>Immunization<br/>Dose, mg. (SQ)<br/>Reaction<br/>Antibody Titer<br/>Hemagglutination<br/>Gel-Diffusion<br/>Intravenous Challenge<br/>Dose (mcg./kg.)<br/>Reaction</p> | 0.25<br>N.R. | 40,000<br>No Zone | 0.25<br>N.R. | 10,000<br>Positive | 0.25<br>N.R. | 640,000<br>No Zone  | 1000<br>I    |  |

Sample #27

Sample #27

Exposure to ultraviolet light for 5 hours

Date

|  | 9/12/63      | 10/8/63            | 10/17/63     | 11/11/63          | 11/20/63     | 12/2/63            | 12/10/63     |
|--|--------------|--------------------|--------------|-------------------|--------------|--------------------|--------------|
| <u>Monkey #28</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>N.R. | <10,000<br>No Zone | 0.50<br>N.R. | 10,000<br>No Zone | 0.25<br>N.R. | 320,000<br>No Zone | 10.0<br>N.R. |
|  | 0.25<br>N.R. | 80,000<br>No Zone  | 0.50<br>N.R. | 40,000<br>No Zone | 0.25<br>N.R. | 320,000<br>No Zone | 1000<br>I    |
| <u>Monkey #29</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>N.R. | <10,000<br>No Zone | 0.50<br>N.R. | 10,000<br>No Zone | 0.25<br>N.R. | 320,000<br>No Zone | 10.0<br>N.R. |
|  | 0.25<br>N.R. | 80,000<br>No Zone  | 0.50<br>N.R. | 40,000<br>No Zone | 0.25<br>N.R. | 320,000<br>No Zone | 1000<br>I    |



sample #20

Photodynamic action of methylene blue - 1 hour

Date

|  | 9/12/63      | 10/8/63            | 10/17/63     | 11/11/63          | 11/20/63     | 12/2/63             | 12/10/63     |
|--|--------------|--------------------|--------------|-------------------|--------------|---------------------|--------------|
| <b>Monkey #30</b><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I    | 160,000<br>No Zone | 0.25<br>N.R. | 40,000<br>No Zone | 0.25<br>N.R. | 320,000<br>Positive | 10.0<br>N.R. |
| <b>Monkey #31</b><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.25<br>N.R. | 10,000<br>No Zone  | 0.25<br>N.R. | 20,000<br>No Zone | 0.25<br>N.R. | 160,000<br>No Zone  | 1000<br>N.R. |

Sample #29

Photodynamic action of methylene blue - 8 hours

Date

|  | 9/12/63   | 10/8/63            | 10/17/63     | 11/11/63           | 11/20/63     | 12/2/63             | 12/10/63     |
|--|-----------|--------------------|--------------|--------------------|--------------|---------------------|--------------|
| <u>Monkey #26</u><br>Immunization<br>Dose, mg. (sq)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I | <10,000<br>No Zone | 0.25<br>N.R. | <10,000<br>No Zone | 0.25<br>N.R. | 40,000<br>No Zone   | 10.0<br>N.R. |
|  |           |                    |              |                    |              | 160,000<br>Positive | 1000<br>I    |
| <u>Monkey #27</u><br>Immunization<br>Dose, mg. (sq)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.25<br>I | <10,000<br>No Zone | 0.25<br>N.R. | 10,000<br>No Zone  | 0.25<br>N.R. |                     |              |
|  |           |                    |              |                    |              |                     |              |

Sample #30

Acetylation with acetic anhydride

Date

|  | 9/12/63   | 10/8/63           | 10/17/63     | 11/11/63          | 11/20/63     | 12/3/63             | 12/10/63     |  |
|--|-----------|-------------------|--------------|-------------------|--------------|---------------------|--------------|--|
| <u>Monkey #38</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I | 10,000<br>No Zone | 0.25<br>H.R. | 10,000<br>No Zone | 0.25<br>H.R. | 160,000<br>Positive | 10.0<br>H.R. |  |
|  | 0.25<br>I | 10,000<br>No Zone | 0.25<br>H.R. | 10,000<br>No Zone | 0.25<br>H.R. | 80,000<br>No Zone   | 1000<br>H.R. |  |
| <u>Monkey #39</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I | 10,000<br>No Zone | 0.25<br>H.R. | 10,000<br>No Zone | 0.25<br>H.R. | 160,000<br>Positive | 10.0<br>H.R. |  |
|  | 0.25<br>I | 10,000<br>No Zone | 0.25<br>H.R. | 10,000<br>No Zone | 0.25<br>H.R. | 80,000<br>No Zone   | 1000<br>H.R. |  |

Sample #2

0.2% formalin at 37°C. for 2 weeks

Date

|  | <u>Date</u>  |                     |              |                    |              |                     |
|--|--------------|---------------------|--------------|--------------------|--------------|---------------------|
|  | 12/12/63     | 1/9/64              | 1/16/64      | 2/10/64            | 2/19/64      | 3/4/64              |
| <u>Monkey #1</u><br>Immunization<br>Dose, mg. (S <sub>4</sub> )<br>Reaction*<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mg./kg.)<br>Reaction | 0.10<br>N.R. | 40,000<br>No Zone   | 0.25<br>N.R. | 40,000<br>No Zone  | 0.25<br>N.R. | 80,000<br>No Zone   |
|  |              |                     |              |                    |              | 20,000<br>No Zone   |
| <u>Monkey #2</u><br>Immunization<br>Dose, mg. (S <sub>4</sub> )<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mg./kg.)<br>Reaction  | 0.10<br>I    | < 10,000<br>No Zone | 0.25<br>N.R. | 20,000<br>Positive | 0.25<br>N.R. | 80,000<br>No Zone   |
|  |              |                     |              |                    |              | < 10,000<br>No Zone |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

Sample #2

0.2% formalin at 37°C. for 2 weeks

Date

|  | 12/12/63     | 1/9/64              | 1/16/64      | 2/10/64            | 2/19/64      | 3/14/64             | 5/20/64             |
|--|--------------|---------------------|--------------|--------------------|--------------|---------------------|---------------------|
| <u>Monkey #69</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>N.R. | <10,000<br>No Zone  | 0.25<br>N.R. | 20,000<br>No Zone  | 0.25<br>N.R. | 320,000<br>Positive | < 10,000<br>No Zone |
| <u>Monkey #70</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>I    | < 10,000<br>No Zone | 0.25<br>N.R. | 40,000<br>Positive | 0.25<br>N.R. | 640,000<br>Positive | 40,000<br>No Zone   |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

Sample #2

0.2% formalin at 37°C. for 2 weeks

Date

|   | Date         |                     |              |                     |              |                     |
|---|--------------|---------------------|--------------|---------------------|--------------|---------------------|
|   | 12/12/63     | 1/9/64              | 1/16/64      | 2/10/64             | 2/19/64      | 3/4/64              |
| <p><u>Monkey # 71</u></p> <p>Immunization<br/>Dose, mg. (SQ)<br/>Reaction *</p> <p>Antibody Titer<br/>Hemagglutination<br/>Gel-Diffusion<br/>Intravenous Challenge<br/>Dose (mcg./kg.)<br/>Reaction</p> | 0.10<br>I    | < 10,000<br>No Zone | 0.25<br>N.R. | < 10,000<br>No Zone | 0.25<br>N.R. | 80,000<br>Positive  |
|   |              |                     |              |                     |              | < 10,000<br>No Zone |
| <p><u>Monkey # 72</u></p> <p>Immunization<br/>Dose, mg. (SQ)<br/>Reaction</p> <p>Antibody Titer<br/>Hemagglutination<br/>Gel-Diffusion<br/>Intravenous Challenge<br/>Dose (mcg./kg.)<br/>Reaction</p>   | 0.10<br>N.R. | < 10,000<br>No Zone | 0.25<br>N.R. | 80,000<br>Positive  | 0.25<br>N.R. | 320,000<br>Positive |
|   |              |                     |              |                     |              | 80,000<br>No Zone   |

\* I, Illness (emesis, diarrhea)  
X, Death  
N.R. No Reaction

Sample #2

0.2% formalin at 37°C. for 2 weeks

Date

|  | 12/12/63     | 1/9/64             | 1/16/64      | 2/10/64            | 2/19/64      | 3/4/64              | 5/20/64            |  |
|--|--------------|--------------------|--------------|--------------------|--------------|---------------------|--------------------|--|
| <u>Monkey #73</u><br>Immunization<br>Dose, mg. (Sq)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>N.R. | <10,000<br>No Zone | 0.25<br>N.R. | 80,000<br>Positive | 0.25<br>N.R. | 320,000<br>Positive | 20,000<br>No Zone  |  |
|  | 0.10<br>I    | <10,000<br>No Zone | 0.25<br>N.R. | <10,000<br>No Zone | 0.25<br>N.R. | 20,000<br>No Zone   | <10,000<br>No Zone |  |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

Sample #2

0.2% formalin at 37°C. for 2 weeks

Date

|   | 12/12/63     | 1/9/64              | 1/16/64      | 2/10/64           | 2/19/64      | 3/4/64            | 5/20/64             |  |
|---|--------------|---------------------|--------------|-------------------|--------------|-------------------|---------------------|--|
| <p><u>Monkey #75</u></p> <p>Immunization<br/>Dose, mg. (SQ)<br/>Reaction *<br/>Antibody Titer<br/>Hemagglutination<br/>Gel-Diffusion<br/>Intravenous Challenge<br/>Dose (mcg./kg.)<br/>Reaction</p> | 0.10<br>I    | < 10,000<br>No Zone | 0.25<br>N.R. | 20,000<br>No Zone | 0.25<br>N.R. | 80,000<br>No Zone | < 10,000<br>No Zone |  |
|   | 0.10<br>N.R. | < 10,000<br>No Zone | 0.25<br>N.R. | 10,000<br>No Zone | 0.25<br>N.R. | 20,000<br>No Zone | < 10,000<br>No Zone |  |

\* I, Illness (emesis, diarrhea)  
X, Death  
N.R. No Reaction



Sample #2

0.2% formalin at 37°C. for 2 weeks

Date

|  | 12/12/63     | 1/9/64              | 1/16/64      | 2/10/64            | 2/19/64      | 3/4/64              | 5/20/64            |  |
|--|--------------|---------------------|--------------|--------------------|--------------|---------------------|--------------------|--|
| <u>Monkey #77</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I    | 10,000<br>No Zone   | 0.25<br>N.R. | 80,000<br>Positive | 0.25<br>N.R. | 320,000<br>Positive | 160,000<br>No Zone |  |
| <u>Monkey #78</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>N.R. | 160,000<br>Positive | 0.25<br>N.R. | 1,280,000<br>220   | 0.25<br>N.R. | 2,560,000<br>240    | 640,000<br>No Zone |  |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

Sample #10

0.2% formalin + 0.15% glycine at 25°C. for 2 weeks

Date

|  | 1/14/64      | 2/10/64            | 2/17/64      | 3/17/64          | 3/24/64      | 4/7/64              | 6/24/64                 |
|--|--------------|--------------------|--------------|------------------|--------------|---------------------|-------------------------|
| <b>Monkey # 152</b><br>Immunization<br>Dose, mg. (SQ)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>N.R. | 160,000<br>No Zone | 0.25<br>N.R. | 1,280,000<br>145 | 0.25<br>N.R. | 640,000<br>Positive | 160,000<br>No Zone      |
| <b>Monkey # 153</b><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>N.R. | 40,000<br>Positive | 0.25<br>N.R. | 640,000<br>235   | 0.25<br>N.R. | 1,280,000<br>870    | 1,280,000<br>Positive** |

\* I, Illness (anemia, diarrhea)  
 X, Death  
 N.R. No Reaction

\*\* Quantitative titer to be determined

Sample #10

0.2% formalin + 0.15% glycine at 25° C. for 2 weeks

Date

|  | 1/14/64      | 2/10/64             | 2/17/64      | 3/17/64             | 3/24/64      | 4/7/64              | 6/24/64             |  |
|--|--------------|---------------------|--------------|---------------------|--------------|---------------------|---------------------|--|
| <b>Monkey # 154</b><br>Immunization<br>Dose, mg. (SQ)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>N.R. | < 10,000<br>No Zone | 0.25<br>N.R. | 160,000<br>No Zone  | 0.25<br>N.R. | 160,000<br>No Zone  | 160,000<br>No Zone  |  |
|  | 0.10<br>N.R. | < 10,000<br>No Zone | 0.25<br>N.R. | 320,000<br>Positive | 0.25<br>N.R. | 320,000<br>Positive | 320,000<br>Positive |  |
| <b>Monkey # 155</b><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>N.R. | < 10,000<br>No Zone | 0.25<br>N.R. | 320,000<br>Positive | 0.25<br>N.R. | 320,000<br>Positive | 320,000<br>Positive |  |
|  | 0.10<br>N.R. | < 10,000<br>No Zone | 0.25<br>N.R. | 320,000<br>Positive | 0.25<br>N.R. | 320,000<br>Positive | 320,000<br>Positive |  |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

Sample #10

0.2% formalin + 0.15% glycine at 25°C. for 2 weeks

Date

|  | 1/14/64      | 2/10/64             | 2/17/64      | 3/17/64             | 3/24/64      | 4/7/64              | 6/24/64             |  |
|--|--------------|---------------------|--------------|---------------------|--------------|---------------------|---------------------|--|
| <b>Monkey # 154</b><br>Immunization<br>Dose, mg. (SQ)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>N.R. | < 10,000<br>No Zone | 0.25<br>N.R. | 160,000<br>No Zone  | 0.25<br>N.R. | 160,000<br>No Zone  | 160,000<br>No Zone  |  |
| <b>Monkey # 155</b><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>N.R. | < 10,000<br>No Zone | 0.25<br>N.R. | 320,000<br>Positive | 0.25<br>N.R. | 320,000<br>Positive | 320,000<br>Positive |  |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

Sample #10

0.2% formalin + 0.15% glycine at 25°C. for 2 weeks

Date

|  | 1/14/64      | 2/10/64             | 2/17/64      | 3/17/64              | 3/24/64      | 4/7/64              | 6/24/64           |  |
|--|--------------|---------------------|--------------|----------------------|--------------|---------------------|-------------------|--|
| <u>Monkey # 156</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>N.R. | 10,000<br>No Zone   | 0.25<br>N.R. | 40,000<br>Positive   | 0.25<br>N.R. | 640,000<br>Positive | 80,000<br>No Zone |  |
| <u>Monkey # 157</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>N.R. | < 10,000<br>No Zone | 0.25<br>N.R. | < 10,000<br>Positive | 0.25<br>N.R. | 20,000<br>Positive  | 10,000<br>No Zone |  |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

Sample #10

0.2% formalin + 0.15% glycine at 25°C. for 2 weeks

Date

|  | 1/14/64      | 2/12/64             | 2/17/64      | 3/17/64            | 3/24/64      | 3/24/64             | 4/7/64                | 6/24/64 |
|--|--------------|---------------------|--------------|--------------------|--------------|---------------------|-----------------------|---------|
| <b>Monkey # 158</b><br>Immunization<br>Dose, mg. (Sq)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>N.R. | < 10,000<br>No Zone | 0.25<br>N.R. | 10,000<br>Positive | 0.25<br>N.R. | 160,000<br>Positive | 80,000<br>No Zone     |         |
| <b>Monkey # 159</b><br>Immunization<br>Dose, mg. (Sq)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>N.R. | 160,000<br>No Zone  | 0.25<br>N.R. | 640,000<br>175     | 0.25<br>N.R. | 1,280,000<br>145    | 640,000<br>Positive** |         |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

\*\* Quantitative titer to be determined

Sample #10

0.2% formalin + 0.15% glycine at 25° C. for 2 weeks

Date

|  | 1/14/64      | 2/14/64            | 2/17/64      | 3/17/64             | 3/24/64      | 4/7/64         | 6/24/64                 |
|--|--------------|--------------------|--------------|---------------------|--------------|----------------|-------------------------|
| <u>Monkey # 160</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>N.R. | 20,000<br>Positive | 0.25<br>N.R. | 80,000<br>240       | 0.25<br>N.R. | 160,000<br>455 | 160,000<br>Positive     |
| <u>Monkey # 161</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>N.R. | 40,000<br>No Zone  | 0.25<br>N.R. | 160,000<br>Positive | 0.25<br>N.R. | 640,000<br>480 | 1,280,000<br>Positive** |

\* I, Illness (emesis, diarrhea)

X, Death

N.R. No Reaction

\*\* Quantitative titer to be determined

Sample #10

0.2% formalin + 0.15% glycine at 25° C. for 2 weeks

Date

|  | 1/14/64      | 2/14/64           | 2/17/64      | 3/17/64           | 3/24/64      | 4/7/64            | 6/24/64                 |  |
|--|--------------|-------------------|--------------|-------------------|--------------|-------------------|-------------------------|--|
| <u>Monkey # 162</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>N.R. | 2,560,000<br>400  | 0.25<br>N.R. | 5,120,000<br>1200 | 0.25<br>N.R. | 1,280,000<br>1290 | 2,560,000<br>Positive** |  |
| <u>Monkey # 163</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>N.R. | 10,000<br>No Zone | 0.25<br>N.R. | 80,000<br>275     | 0.25<br>N.R. | 80,000<br>380     | 80,000<br>No Zone       |  |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

\*\* Quantitative titer to be determined



Sample #12

0.3% hexamethylenetetramine at 37°C. for 3 weeks

Date

|  | 12/13/63     | 1/10/64             | 1/17/64      | 2/10/64            | 2/20/64      | 3/5/64              | 5/20/64            |  |
|--|--------------|---------------------|--------------|--------------------|--------------|---------------------|--------------------|--|
| <u>Monkey #81</u><br>Immunization<br>Dose, mg. (sq)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I    | < 10,000<br>No Zone | 0.25<br>N.R. | 40,000<br>Positive | 0.25<br>N.R. | 160,000<br>Positive | 80,000<br>No Zone  |  |
| <u>Monkey #82</u><br>Immunization<br>Dose, mg. (sq)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>N.R. | < 10,000<br>No Zone | 0.25<br>N.R. | 160,000<br>190     | 0.25<br>N.R. | 320,000<br>220      | 320,000<br>No Zone |  |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

Sample #12

0.3% hexamethylenetetramine at 37°C. for 3 weeks

Date

|  | 12/13/63  | 1/10/64            | 1/17/64      | 2/10/64            | 2/20/64      | 3/5/64              | 5/20/64           |  |
|--|-----------|--------------------|--------------|--------------------|--------------|---------------------|-------------------|--|
| <u>Monkey #83</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I | <10,000<br>No Zone | 0.25<br>N.R. | 40,000<br>No Zone  | 0.25<br>N.R. | 150,000<br>Positive | 80,000<br>No Zone |  |
|  | 0.10<br>I | <10,000<br>No Zone | 0.25<br>N.R. | 40,000<br>Positive | 0.25<br>N.R. | 40,000<br>Positive  | 20,000<br>No Zone |  |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

Monkey #84

Immunization  
 Dose, mg. (SQ)  
 Reaction  
 Antibody Titer  
 Hemagglutination  
 Gel-Diffusion  
 Intravenous Challenge  
 Dose (mcg./kg.)  
 Reaction

Sample #12

0.3% hexamethylenetetramine at 37°C. for 3 weeks

Date

|  | 12/13/63     | 1/10/64            | 1/17/64      | 2/10/64            | 2/20/64      | 3/5/64             | 5/20/64               |  |
|--|--------------|--------------------|--------------|--------------------|--------------|--------------------|-----------------------|--|
| <u>Monkey # 85</u><br>Immunization<br>Dose, mg. (SQ)<br>Reactor *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I    | 20,000<br>No Zone  | 0.25<br>H.R. | 40,000<br>Positive | 0.25<br>N.R. | 50,000<br>Positive | 160,000<br>Positive   |  |
|  | 0.10<br>N.R. | <10,000<br>No Zone | 0.25<br>H.R. | 640,000<br>100     | 0.25<br>N.R. | 5,120,000<br>315   | 1,280,000<br>Positive |  |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

Sample #12

0.3% hexamethylenetetramine at 37°C. for 3 weeks

Date

|  | <u>Date</u> |                     |              |                   |              |                    |
|--|-------------|---------------------|--------------|-------------------|--------------|--------------------|
|  | 12/13/53    | 1/10/64             | 2/17/64      | 2/10/64           | 2/20/64      | 3/5/64             |
| <u>Monkey #87</u><br>Immunization<br>Dose, mg. (Sq)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I   | 20,000<br>Positive  | 0.25<br>N.R. | 160,000<br>210    | 0.25<br>N.R. | 5-120,000<br>330   |
|  |             |                     |              |                   |              | 320,000<br>No Zone |
| <u>Monkey #58</u><br>Immunization<br>Dose, mg. (Sq)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>I   | < 10,000<br>No Zone | 0.25<br>N.R. | 40,000<br>No Zone | 0.25<br>N.R. | 80,000<br>No Zone  |
|  |             |                     |              |                   |              | 40,000<br>No Zone  |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

Sample #12

0.3% hexamethylene tetramine at 37° C. for 3 weeks

Date

|   | 12/13/53     | 1/10/54             | 1/17/64      | 2/10/64             | 2/20/64      | 3/5/64              | 5/20/64            |  |
|---|--------------|---------------------|--------------|---------------------|--------------|---------------------|--------------------|--|
| <u>Monkey # 89</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>N.R. | 160,000<br>275      | 0.25<br>N.R. | 1,280,000<br>300    | 0.25<br>N.R. | 1,280,000<br>315    | 640,000<br>No Zone |  |
| <u>Monkey # 91</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.1<br>I     | < 10,000<br>No Zone | 0.25<br>N.R. | 160,000<br>Positive | 0.25<br>N.R. | 640,000<br>Positive | 320,000<br>No Zone |  |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

Sample #12

0.3% hexamethylenetetramine at 37°C. for 3 weeks

Date

|  | 12/13/63     | 1/10/64              | 1/17/64      | 2/10/64            | 2/20/64      | 3/5/64             | 5/20/64            |  |
|--|--------------|----------------------|--------------|--------------------|--------------|--------------------|--------------------|--|
| <u>Monkey #92</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>N.R. | < 10,000<br>Positive | 0.25<br>N.R. | 40,000<br>Positive | 0.25<br>N.R. | 40,000<br>Positive | 160,000<br>No Zone |  |
| <u>Monkey #93</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>I    | < 10,000<br>No Zone  | 0.25<br>N.R. | ---                | 0.25<br>N.R. | 80,000<br>No Zone  | 40,000<br>No Zone  |  |

\* I, Illness (emesis, diarrhea)  
 A, Death  
 N.R. No Reaction

Date

| <p>Monkey # 156</p> <p>Immunization<br/>Dose, mg. (SQ)<br/>Reaction *<br/>Antibody Titer<br/>Hemagglutination<br/>Gel-Diffusion<br/>Intravenous Challenge<br/>Dose (mcg./kg.)<br/>Reaction</p> | 1/15/54   | 2/14/54             | 2/18/54      | 3/15/54             | 3/25/54      | 4/8/54             | 6/25/64            |
|--|-----------|---------------------|--------------|---------------------|--------------|--------------------|--------------------|
|  | 0.10<br>I | < 10,000<br>No Zone | 0.25<br>N.R. | < 10,000<br>No Zone | 0.25<br>N.R. | 40,000<br>Positive | 80,000<br>No Zone  |
| <p>Monkey # 157</p> <p>Immunization<br/>Dose, mg. (SQ)<br/>Reaction<br/>Antibody Titer<br/>Hemagglutination<br/>Gel-Diffusion<br/>Intravenous Challenge<br/>Dose (mcg./kg.)<br/>Reaction</p>   | 0.10<br>I | < 10,000<br>No Zone | 0.25<br>N.R. | 40,000<br>Positive  | 0.25<br>N.R. | 80,000<br>Positive | 160,000<br>No Zone |

\* I, Illness (emesis, diarrhea)  
X, Death  
N.R. No Reaction

Sample #14

0.2% beta-propiolactone at 37° C. for 2 hours

Date

|   | 1/15/64   | 2/14/64           | 2/18/64      | 3/18/64            | 3/25/64      | 4/8/64              | 6/25/64              |  |
|---|-----------|-------------------|--------------|--------------------|--------------|---------------------|----------------------|--|
| <b>Monkey # 168</b><br>Immunization<br>Dose, mg. (Sq)<br>Reaction *<br>Antibody Titer<br>HemaGlutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I | 40,000<br>No Zone | 0.25<br>N.R. | 40,000<br>Positive | 0.25<br>N.R. | 80,000<br>Positive  | 80,000<br>No Zone    |  |
| <b>Monkey # 169</b><br>Immunization<br>Dose, mg. (Sq)<br>Reaction<br>Antibody Titer<br>HemaGlutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>I | 80,000<br>No Zone | 0.25<br>N.R. | 320,000<br>No Zone | 0.25<br>N.R. | 320,000<br>Positive | 1,280,000<br>No Zone |  |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction





Sample #14

0.2% beta-propiolactone at 37° C. for 2 hours

Date

|   | 1/15/64   | 2/14/64             | 2/18/64      | 3/18/64            | 3/23/64      | 4/8/64              | 6/25/64            |  |
|---|-----------|---------------------|--------------|--------------------|--------------|---------------------|--------------------|--|
| <b>Monkey #172</b><br>Immunization<br>Dose, mg. (SQ)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I | 160,000<br>No Zone  | 0.25<br>N.R. | 40,000<br>No Zone  | 0.25<br>N.R. | 160,000<br>Positive | 160,000<br>No Zone |  |
| <b>Monkey #173</b><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>I | < 10,000<br>No Zone | 0.25<br>N.R. | 40,000<br>Positive | 0.25<br>N.R. | 320,000<br>Positive | 320,000<br>No Zone |  |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

Sample #14

0.2% beta-propiolactone at 37°C. for 2 hours

Date

|   | 1/15/64   | 2/14/64             | 2/18/64      | 3/18/64           | 3/25/64      | 4/8/64             | 6/25/64           |  |
|---|-----------|---------------------|--------------|-------------------|--------------|--------------------|-------------------|--|
| <u>Monkey #174</u><br>Immunization<br>Dose, mg. (Sq)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I | < 10,000<br>No Zone | 0.25<br>N.R. | 40,000<br>No Zone | 0.25<br>N.R. | 40,000<br>No Zone  | 40,000<br>No Zone |  |
| <u>Monkey #175</u><br>Immunization<br>Dose, mg. (Sq)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>I | 40,000<br>No Zone   | 0.25<br>N.R. | 80,000<br>No Zone | 0.25<br>N.R. | 160,000<br>No Zone | 40,000<br>No Zone |  |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

Sample #14

0.2% beta-propiolactone at 37°C. for 2 hours

Date

|                             | 2/15/64 | 2/14/64  | 2/18/64 | 3/18/64  | 3/25/64 | 4/8/64   | 6/25/64  |  |
|-----------------------------|---------|----------|---------|----------|---------|----------|----------|--|
| Monkey # 175                |         |          |         |          |         |          |          |  |
| Immunization                |         |          |         |          |         |          |          |  |
| Dose, mg. (S <sub>2</sub> ) | 0.10    |          | 0.25    |          | 0.25    |          |          |  |
| Reaction *                  | I       |          | N.R.    |          | N.R.    |          |          |  |
| Antibody Titer              |         |          |         |          |         |          |          |  |
| Hemagglutination            |         |          |         |          |         |          |          |  |
| Gel-Diffusion               |         | < 10,000 |         | < 10,000 |         | < 10,000 | < 10,000 |  |
| Intravenous Challenge       |         | No Zone  |         | No Zone  |         | No Zone  | No Zone  |  |
| Dose (mcg./kg.)             |         |          |         |          |         |          |          |  |
| Reaction                    |         |          |         |          |         |          |          |  |
| Monkey # 177                |         |          |         |          |         |          |          |  |
| Immunization                |         |          |         |          |         |          |          |  |
| Dose, mg. (S <sub>2</sub> ) | 0.10    |          | 0.25    |          | 0.25    |          |          |  |
| Reaction                    | I       |          | N.R.    |          | N.R.    |          |          |  |
| Antibody Titer              |         |          |         |          |         |          |          |  |
| Hemagglutination            |         |          |         |          |         |          |          |  |
| Gel-Diffusion               |         | < 10,000 |         | 320,000  |         | 320,000  | 320,000  |  |
| Intravenous Challenge       |         | No Zone  |         | No Zone  |         | Positive | No Zone  |  |
| Dose (mcg./kg.)             |         |          |         |          |         |          |          |  |
| Reaction                    |         |          |         |          |         |          |          |  |

\* I, Illness (emesis, diarrhea)  
X, Death  
N.R. No Reaction

0.2% diethylpyrocabonate at 5°C. for 1 week

Date

|  | 1/5/64    | 1/31/64             | 2/10/64      | 3/10/64             | 3/16/64      | 3/30/64            | 6/16/64           |  |  |
|--|-----------|---------------------|--------------|---------------------|--------------|--------------------|-------------------|--|--|
| <u>Monkey #96</u><br>Immunization<br>Dose, mg. (Sq)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I | < 10,000<br>No Zone | 0.25<br>N.R. | < 10,000<br>No Zone | 0.25<br>N.R. | 160,000<br>No Zone | 20,000<br>No Zone |  |  |
|  |           |                     |              |                     |              |                    |                   |  |  |
|  |           |                     |              |                     |              |                    |                   |  |  |
|  |           |                     |              |                     |              |                    |                   |  |  |
| <u>Monkey #97</u><br>Immunization<br>Dose, mg. (Sq)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>I | < 10,000<br>No Zone | 0.25<br>N.R. | < 10,000<br>No Zone | 0.25<br>N.R. | 160,000<br>No Zone | 40,000<br>No Zone |  |  |
|  |           |                     |              |                     |              |                    |                   |  |  |
|  |           |                     |              |                     |              |                    |                   |  |  |
|  |           |                     |              |                     |              |                    |                   |  |  |

\* I, Illness (emesis, diarrhea)

X, Death

N.R. No Reaction

Sample #16

0.2% diethylpyrocarbonate at 5°C. for 1 week

Date

|   | <u>Date</u> |                   |              |                   |              |                   |
|---|-------------|-------------------|--------------|-------------------|--------------|-------------------|
|   | 2/6/64      | 1/31/64           | 2/10/64      | 3/10/64           | 3/16/64      | 3/30/64           |
| <u>Monkey # 98</u><br>Immunization<br>Dose, mg. (Sq)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I   | 10,000<br>No Zone | 0.25<br>H.R. | 10,000<br>No Zone | 0.25<br>N.R. | 80,000<br>No Zone |
|   | 0.10<br>X   |                   |              |                   |              | 40,000<br>No Zone |
| <u>Monkey #99</u><br>Immunization<br>Dose, mg. (Sq)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction    |             |                   |              |                   |              |                   |
|   |             |                   |              |                   |              |                   |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

Sample #16

0.2% diethylpyrocarbonate at 5°C. for 1 week

Date

|   | 1/6/64    | 1/31/64             | 2/10/64      | 3/10/64             | 3/16/64      | 3/30/64           | 6/16/64             |  |
|---|-----------|---------------------|--------------|---------------------|--------------|-------------------|---------------------|--|
| <p><u>Monkey # 100</u></p> <p>Immunization</p> <p>Dose, mg. (Sq)</p> <p>Reaction *</p> <p>Antibody Titer</p> <p>Hemagglutination</p> <p>Gel-Diffusion</p> <p>Intravenous Challenge</p> <p>Dose (mcg./kg.)</p> <p>Reaction</p> | 0.10<br>I | < 10,000<br>No Zone | 0.25<br>N.R. | < 10,000<br>No Zone | 0.25<br>N.R. | 40,000<br>No Zone | < 10,000<br>No Zone |  |
| <p><u>Monkey # 101</u></p> <p>Immunization</p> <p>Dose, mg. (Sq)</p> <p>Reaction</p> <p>Antibody Titer</p> <p>Hemagglutination</p> <p>Gel-Diffusion</p> <p>Intravenous Challenge</p> <p>Dose (mcg./kg.)</p> <p>Reaction</p>   | 0.10<br>I | < 10,000<br>No Zone | 0.25<br>N.R. | < 10,000<br>No Zone | 0.25<br>N.R. | 40,000<br>No Zone | 20,000<br>No Zone   |  |

\* I, Illness (emesis, diarrhea)  
X, Death  
N.R. No Reaction

Sample #16

0.2% diethylpyrocarbonate at 5°C. for 1 week

Date

|  | 1/6/64       | 1/31/64             | 2/10/64      | 3/10/64             | 3/16/64      | 3/30/64             | 6/16/64           |  |
|--|--------------|---------------------|--------------|---------------------|--------------|---------------------|-------------------|--|
| <u>Monkey # 102</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>N.R. | 80,000<br>Positive  | 0.25<br>N.R. | 320,000<br>Positive | 0.25<br>N.R. | 2,560,000<br>245    | 80,000<br>No Zone |  |
|  |              |                     |              |                     |              |                     |                   |  |
|  |              |                     |              |                     |              |                     |                   |  |
|  |              |                     |              |                     |              |                     |                   |  |
|  |              |                     |              |                     |              |                     |                   |  |
| <u>Monkey # 103</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>I    | < 10,000<br>No Zone | 0.25<br>N.R. | < 10,000<br>No Zone | 0.25<br>N.R. | 320,000<br>Positive | 40,000<br>No Zone |  |
|  |              |                     |              |                     |              |                     |                   |  |
|  |              |                     |              |                     |              |                     |                   |  |
|  |              |                     |              |                     |              |                     |                   |  |
|  |              |                     |              |                     |              |                     |                   |  |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction



sample #10

0.2% diethylpyrocabonate at 5°C. for 1 week

Date

| Monkey #104 | 1/6/64       | 1/31/64             | 2/10/64      | 3/10/64             | 3/16/64      | 3/30/64          | 6/16/64            |  |
|-------------|--------------|---------------------|--------------|---------------------|--------------|------------------|--------------------|--|
|             | 0.10<br>I    | 320,000<br>200      | 0.25<br>N.R. | 640,000<br>Positive | 0.25<br>N.R. | 2,560,000<br>130 | 160,000<br>No Zone |  |
| Monkey #105 | 1/6/64       | 1/31/64             | 2/10/64      | 3/10/64             | 3/16/64      | 3/30/64          | 6/16/64            |  |
|             | 0.10<br>N.R. | < 10,000<br>No Zone | 0.25<br>X    |                     |              |                  |                    |  |

\* I, Illness (emesis, diarrhea)  
X, Death  
N.R. No Reaction

Sample #16

0.2% diethylpyrocabonate at 5°C. for 1 week

Date

|  | 1/6/64    | 1/31/64             | 2/10/64      | 3/10/64            | 3/16/64      | 3/30/64            | 6/16/64           |  |
|--|-----------|---------------------|--------------|--------------------|--------------|--------------------|-------------------|--|
| <b>Monkey # 106</b><br>Immunization<br>Dose, mg. (SQ)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>X |                     |              |                    |              |                    |                   |  |
| <b>Monkey # 107</b><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>I | < 10,000<br>No Zone | 0.25<br>N.R. | <10,000<br>No Zone | 0.25<br>N.R. | 160,000<br>No Zone | 10,000<br>No Zone |  |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

0.2% ethylene oxide at 5 °C. for 2 hours

Date

|  | 1/20/64   | 2/14/64            | 2/24/64      | 3/19/64            | 3/30/64      | 4/13/64            | 6/30/64           |  |
|--|-----------|--------------------|--------------|--------------------|--------------|--------------------|-------------------|--|
| <u>Monkey # 180</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I | <10,000<br>No Zone | 0.25<br>N.R. | 20,000<br>No Zone  | 0.25<br>N.R. | 40,000<br>Positive | 20,000<br>No Zone |  |
| <u>Monkey # 181</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>I | <10,000<br>No Zone | 0.25<br>N.R. | 80,000<br>Positive | 0.25<br>N.R. | 80,000<br>Positive | 80,000<br>No Zone |  |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

Sample #18

0.2% ethylene oxide at 5°C. for 2 hours

Date

|  | 1/20/64      | 2/15/64             | 2/24/64      | 3/19/64            | 3/30/64      | 4/13/64            | 6/30/64             |  |
|--|--------------|---------------------|--------------|--------------------|--------------|--------------------|---------------------|--|
| <u>Monkey #182</u><br>Immunization<br>Dose, mg. (Eq)<br>Reaction*<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I    | < 10,000<br>No Zone | 0.25<br>N.R. | 10,000<br>No Zone  | 0.25<br>N.R. | 20,000<br>Positive | 80,000<br>Positive  |  |
| <u>Monkey #183</u><br>Immunization<br>Dose, mg. (Eq)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction  | 0.10<br>N.R. | 10,000<br>Positive  | 0.25<br>N.R. | 40,000<br>Positive | 0.25<br>N.R. | 40,000<br>Positive | 160,000<br>Positive |  |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

0.2% ethylene oxide at 5°C. for 2 hours

Date

|  | 1/20/64   | 2/14/64             | 2/24/64      | 3/19/64            | 3/30/64      | 4/13/64            | 6/30/64           |  |
|--|-----------|---------------------|--------------|--------------------|--------------|--------------------|-------------------|--|
| <u>Monkey # 184</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I | < 10,000<br>No Zone | 0.25<br>N.R. | 40,000<br>Positive | 0.25<br>N.R. | 40,000<br>Positive | 80,000<br>No Zone |  |
| <u>Monkey # 185</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>I | < 10,000<br>No Zone | X            |                    |              |                    |                   |  |

\* I, Illness (emesis, diarrhea)  
X, Death

N.R. No Reaction

Sample #18

0.2% ethylene oxide at 5 °C. for 2 hours

Date

|   | 1/20/64      | 2/14/64              | 2/24/64      | 3/19/64           | 3/30/64      | 4/13/64            | 6/30/64             |
|---|--------------|----------------------|--------------|-------------------|--------------|--------------------|---------------------|
| <u>Monkey # 186</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction *<br>Antibody Titer<br>HemaGlutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>N.R. | < 10,000<br>Positive | 0.25<br>N.R. | 40,000<br>No Zone | 0.25<br>N.R. | 20,000<br>Positive | 640,000<br>Positive |
| <u>Monkey # 187</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>HemaGlutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>I    | < 10,000<br>No Zone  | 0.25<br>N.R. | 40,000<br>No Zone | 0.25<br>N.R. | 40,000<br>Positive | 20,000<br>No Zone   |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

Sample #18

0.2% ethylene oxide at 5°C. for 2 hours

Date

|   | 1/20/64   | 2/14/64            | 2/24/64      | 3/19/64             | 3/30/64      | 4/13/64            | 6/30/64            |
|---|-----------|--------------------|--------------|---------------------|--------------|--------------------|--------------------|
| <p>Monkey # 188</p> <p>Immunization<br/>Dose, mg. (SQ)<br/>Reaction *</p> <p>Antibody Titer<br/>Hemagglutination<br/>Gel-Diffusion<br/>Intravenous Challenge<br/>Dose (mcg./kg.)<br/>Reaction</p> | 0.10<br>I | 80,000<br>Positive | 0.25<br>N.R. | 160,000<br>Positive | 0.25<br>N.R. | 160,000<br>80      | 320,000<br>No Zone |
| <p>Monkey # 189</p> <p>Immunization<br/>Dose, mg. (SQ)<br/>Reaction</p> <p>Antibody Titer<br/>Hemagglutination<br/>Gel-Diffusion<br/>Intravenous Challenge<br/>Dose (mcg./kg.)<br/>Reaction</p>   | 0.10<br>I | 40,000<br>Positive | 0.25<br>N.R. | 40,000<br>No Zone   | 0.25<br>N.R. | 80,000<br>Positive | 20,000<br>No Zone  |

\* I, Illness (emesis, diarrhea)  
X, Death  
N.R. No Reaction

Sample #18

0.2% ethylene oxide at 5°C. for 2 hours

Date

|  | 1/20/64   | 2/14/64                | 2/24/64      | 3/19/64           | 3/30/64      | 4/13/64           | 6/30/64           |  |
|--|-----------|------------------------|--------------|-------------------|--------------|-------------------|-------------------|--|
| <p><u>Monkey # 190</u></p> <p>Immunization<br/>Dose, mg. (SQ)<br/>Reaction *</p> <p>Antibody Titer<br/>Hemagglutination<br/>Gel-Diffusion<br/>Intravenous Challenge<br/>Dose (mcg./kg.)<br/>Reaction</p> | 0.10<br>I | 10,000<br>No Zone      | 0.25<br>N.R. | 40,000<br>No Zone | 0.25<br>N.R. | 20,000<br>No Zone | 40,000<br>No Zone |  |
| <p><u>Monkey # 191</u></p> <p>Immunization<br/>Dose, mg. (SQ)<br/>Reaction</p> <p>Antibody Titer<br/>Hemagglutination<br/>Gel-Diffusion<br/>Intravenous Challenge<br/>Dose (mcg./kg.)<br/>Reaction</p>   | 0.10<br>I | X<br>10,000<br>No Zone |              |                   |              |                   |                   |  |

\* I, Illness (emesis, diarrhea)  
X, Death  
N.R. No Reaction



Sample #22

Exposure to methyl bromide vapor for 24 hours

Date

|  | 1/21/54   | 2/14/54             | 2/25/54      | 3/20/54           | 3/31/54      | 4/14/54             | 7/1/54              |
|--|-----------|---------------------|--------------|-------------------|--------------|---------------------|---------------------|
| <u>Monkey # 194</u><br>Immunization<br>Dose, mg. (Sq)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I | < 10,000<br>No Zone | 0.25<br>N.R. | 20,000<br>No Zone | 0.25<br>N.R. | 40,000<br>Positive  | 20,000<br>No Zone   |
| <u>Monkey # 195</u><br>Immunization<br>Dose, mg. (Sq)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>I | < 10,000<br>No Zone | 0.25<br>N.R. | 20,000<br>No Zone | 0.25<br>N.R. | < 10,000<br>No Zone | < 10,000<br>No Zone |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

Sample #22

Exposure to methyl bromide vapor for 24 hours

Date

|  | 1/21/64      | 2/14/64             | 2/25/64      | 3/20/64             | 3/31/64      | 4/14/64             | 7/1/64            |  |
|--|--------------|---------------------|--------------|---------------------|--------------|---------------------|-------------------|--|
| <u>Monkey # 196</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>N.R. | 320,000<br>Positive | 0.25<br>N.R. | 160,000<br>Positive | 0.25<br>N.R. | 160,000<br>Positive | 40,000<br>No Zone |  |
|  | 0.10<br>N.R. | 10,000<br>No Zone   | 0.25<br>N.R. | 160,000<br>Positive | 0.25<br>N.R. | 320,000<br>Positive |                   |  |
| <u>Monkey # 197</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>N.R. | 10,000<br>No Zone   | 0.25<br>N.R. | 160,000<br>Positive | 0.25<br>N.R. | 320,000<br>Positive |                   |  |
|  | 0.10<br>N.R. | 10,000<br>No Zone   | 0.25<br>N.R. | 160,000<br>Positive | 0.25<br>N.R. | 320,000<br>Positive |                   |  |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

Exposure to methyl bromide vapor for 24 hours

Date

|   | 1/21/64      | 2/14/64             | 2/25/64      | 3/20/64             | 3/31/64      | 4/10/64             | 7/1/64            |
|---|--------------|---------------------|--------------|---------------------|--------------|---------------------|-------------------|
| <p><u>Monkey # 200</u></p> <p>Immunization<br/>Dose, mg. (Sq)<br/>Reaction *<br/>Antibody Titer<br/>Hemagglutination<br/>Gel-Diffusion<br/>Intravenous Challenge<br/>Dose (m 3./kg.)<br/>Reaction</p> | 0.10<br>I    | < 10,000<br>No Zone | 0.25<br>N.R. | < 10,000<br>No Zone | 0.25<br>N.R. | X                   |                   |
| <p><u>Monkey # 201</u></p> <p>Immunization<br/>Dose, mg. (Sq)<br/>Reaction<br/>Antibody Titer<br/>Hemagglutination<br/>Gel-Diffusion<br/>Intravenous Challenge<br/>Dose (mcg./kg.)<br/>Reaction</p>   | 0.10<br>N.R. | 160,000<br>No Zone  | 0.25<br>N.R. | 160,000<br>No Zone  | 0.25<br>N.R. | 160,000<br>Positive | 80,000<br>No Zone |

\* I, Illness (emesis, diarrhea)  
X, Death  
N.R. No Reaction

Sample #22

Exposure to methyl bromide vapor for 24 hours

Date

|  | 1/21/64      | 2/14/64             | 2/25/64      | 3/20/64           | 3/31/64      | 4/14/64           | 7/1/64              |  |
|--|--------------|---------------------|--------------|-------------------|--------------|-------------------|---------------------|--|
| <p><u>Monkey # 202</u></p> <p>Immunization<br/>Dose, mg. (SQ)<br/>Reaction *</p> <p>Antibody Titer<br/>Hemagglutination<br/>Gel-Diffusion<br/>Intravenous Challenge<br/>Dose (mcg./kg.)<br/>Reaction</p> | 0.10<br>I    | 10,000<br>No Zone   | 0.25<br>N.R. | 20,000<br>No Zone | 0.25<br>N.R. | 160,000<br>70     | 80,000<br>No Zone   |  |
| <p><u>Monkey # 203</u></p> <p>Immunization<br/>Dose, mg. (SQ)<br/>Reaction</p> <p>Antibody Titer<br/>Hemagglutination<br/>Gel-Diffusion<br/>Intravenous Challenge<br/>Dose (mcg./kg.)<br/>Reaction</p>   | 0.10<br>N.R. | < 10,000<br>No Zone | 0.25<br>N.R. | 10,000<br>No Zone | 0.25<br>N.R. | 20,000<br>No Zone | < 10,000<br>No Zone |  |

\* I, Illness (emesis, diarrhea)  
X, Death  
N.R. No Reaction

Sample #22

Exposure to methyl bromide vapor for 24 hours

Date

| <p> <u>Monkey # 204</u><br/>           Immunization<br/>           Dose, mg. (S<sub>2</sub>)<br/>           Reaction *<br/>           Antibody Titer<br/>           Hemagglutination<br/>           Gel-Diffusion<br/>           Intravenous Challenge<br/>           Dose (mcg./kg.)<br/>           Reaction         </p> | 1/21/54      | 2/14/54             | 2/25/54      | 3/30/54           | 3/31/54      | 4/14/54           | 7/1/54              |  |
|--|--------------|---------------------|--------------|-------------------|--------------|-------------------|---------------------|--|
|  | 0.10<br>N.R. | 50,000<br>No Zone   | 0.25<br>N.R. | 20,000<br>No Zone | 0.25<br>N.R. | 20,000<br>No Zone | 10,000<br>No Zone   |  |
| <p> <u>Monkey # 205</u><br/>           Immunization<br/>           Dose, mg. (S<sub>2</sub>)<br/>           Reaction<br/>           Antibody Titer<br/>           Hemagglutination<br/>           Gel-Diffusion<br/>           Intravenous Challenge<br/>           Dose (mcg./kg.)<br/>           Reaction         </p>   | 0.10<br>I    | < 10,000<br>No Zone | 0.25<br>N.R. | 20,000<br>No Zone | 0.25<br>N.R. | 20,000<br>No Zone | < 10,000<br>No Zone |  |
|  |              |                     |              |                   |              |                   |                     |  |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R., No Reaction

Sample #25

1.0% sodium gluconate at 37°C. for 4 weeks

Date

|   | <u>Date</u> |                    |                           |                    |              |                         |
|---|-------------|--------------------|---------------------------|--------------------|--------------|-------------------------|
|   | 1/13/64     | 2/10/64            | 2/17/64                   | 3/16/64            | 3/23/64      | 4/5/64                  |
| <u>Monkey #138</u><br>Immunization<br>Dose, mg. (Sq)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I   | <10,000<br>No Zone | 0.25 <sup>+</sup><br>N.R. | 40,000<br>Positive | 0.25<br>N.R. | 1,280,000<br>235        |
|   |             |                    |                           |                    |              | 2,560,000<br>Positive** |
| <u>Monkey #139</u><br>Immunization<br>Dose, mg. (Sq)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>I   | 10,000<br>No Zone  | 0.25<br>N.R.              | 20,000<br>Positive | 0.25<br>N.R. | 640,000<br>125          |
|   |             |                    |                           |                    |              | 160,000<br>No Zone      |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

\*\* Quantitative titer to be determined

1.0% sodium gluconate at 37°C. for 4 weeks

Date

|  | 1/13/64   | 2/10/64             | 2/17/64      | 3/16/64           | 3/23/64      | 4/6/64             | 6/23/64           |  |
|--|-----------|---------------------|--------------|-------------------|--------------|--------------------|-------------------|--|
| <u>Monkey # 140</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I | < 10,000<br>No Zone | 0.25<br>N.R. | 80,000<br>No Zone | 0.25<br>N.R. | 640,000<br>No Zone | 40,000<br>No Zone |  |
| <u>Monkey # 141</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>I | < 10,000<br>No Zone | 0.25<br>N.R. | 20,000<br>No Zone | 0.25<br>N.R. | 80,000<br>No Zone  | 80,000<br>No Zone |  |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

Sample #25

1.0% sodium gluconate at 37° C. for 4 weeks

Date

|  | 1/13/64      | 2/10/64            | 2/17/64      | 3/16/64               | 3/23/64      | 4/6/64         | 6/23/64             |  |
|--|--------------|--------------------|--------------|-----------------------|--------------|----------------|---------------------|--|
| <u>Monkey #142</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction *<br>Antibody Titer<br>HemaGlutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I    | <10,000<br>No Zone | 0.25<br>N.R. | 160,000<br>Positive   | 0.25<br>N.R. | 640,000<br>190 | 640,000<br>Positive |  |
| <u>Monkey #143</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>HemaGlutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>N.R. | 40,000<br>No Zone  | 0.25<br>N.R. | 1,280,000<br>Positive | 0.25<br>N.R. | 640,000<br>160 | 320,000<br>No Zone  |  |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction



1.0% sodium gluconate at 37°C. for 4 weeks

Date

|   | 1/13/64   | 2/10/64             | 2/17/64      | 3/16/64            | 3/23/64      | 4/6/64                | 6/23/64            |  |
|---|-----------|---------------------|--------------|--------------------|--------------|-----------------------|--------------------|--|
| <u>Monkey # 144</u><br>Immunization<br>Dose, mg. (S <sub>2</sub> )<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I | < 10,000<br>No Zone | 0.25<br>N.R. | 80,000<br>No Zone  | 0.25<br>N.R. | 640,000<br>160        | 640,000<br>No Zone |  |
| <u>Monkey # 145</u><br>Immunization<br>Dose, mg. (S <sub>2</sub> )<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>I | < 10,000<br>No Zone | 0.25<br>N.R. | 160,000<br>No Zone | 0.25<br>N.R. | 1,280,000<br>Positive | 40,000<br>No Zone  |  |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

Sample #25

1.0% sodium gluconate at 37°C. for 4 weeks

Date

|  | 1/13/64   | 2/10/64             | 2/17/64      | 3/16/64            | 3/23/64      | 4/5/64             | 6/23/64             |  |
|--|-----------|---------------------|--------------|--------------------|--------------|--------------------|---------------------|--|
| <u>Monkey # 146</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I | < 10,000<br>No Zone | 0.25<br>N.R. | 80,000<br>Positive | 0.25<br>N.R. | 1,280,000<br>190   | 640,000<br>Positive |  |
|  | 0.10<br>I | < 10,000<br>No Zone | 0.25<br>N.R. | 40,000<br>No Zone  | 0.25<br>N.R. | 320,000<br>No Zone | 160,000<br>No Zone  |  |
| <u>Monkey # 147</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>I | < 10,000<br>No Zone | 0.25<br>N.R. | 40,000<br>No Zone  | 0.25<br>N.R. | 320,000<br>No Zone | 160,000<br>No Zone  |  |
|  | 0.10<br>I | < 10,000<br>No Zone | 0.25<br>N.R. | 40,000<br>No Zone  | 0.25<br>N.R. | 320,000<br>No Zone | 160,000<br>No Zone  |  |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

Exposure to ultraviolet light for 1 hour

Date

|   | 1/8/64       | 1/31/64             | 2/12/64      | 3/12/64               | 3/18/64      | 4/1/64              | 6/18/64               |  |
|---|--------------|---------------------|--------------|-----------------------|--------------|---------------------|-----------------------|--|
| <u>Monkey # 124</u><br>Immunization<br>Dose, mg. (S <sub>2</sub> )<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>N.R. | 320,000<br>Positive | 0.25<br>N.R. | 2,550,000<br>Positive | 0.25<br>N.R. | 540,000<br>Positive | 2,550,000<br>Positive |  |
| <u>Monkey # 125</u><br>Immunization<br>Dose, mg. (S <sub>2</sub> )<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>N.R. | < 10,000<br>No Zone | 0.25<br>N.R. | 40,000<br>Positive    | 0.25<br>N.R. | 80,000<br>Positive  | 150,000<br>Positive   |  |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

Sample #26

Exposure to ultraviolet light for 1 hour

Date

|   | 1/8/64       | 1/31/64             | 2/12/64      | 3/12/64             | 3/18/64      | 4/1/64             | 6/18/64             |  |
|---|--------------|---------------------|--------------|---------------------|--------------|--------------------|---------------------|--|
| <p><u>Monkey # 126</u></p> <p>Immunization<br/>Dose, mg. (Sq)<br/>Reaction *<br/>Antibody Titer<br/>Hemagglutination<br/>Gel-Diffusion<br/>Intravenous Challenge<br/>Dose (mcg./kg.)<br/>Reaction</p> | 0.10<br>N.R. | < 10,000<br>No Zone | 0.25<br>N.R. | 160,000<br>Positive | 0.25<br>N.R. | 80,000<br>Positive | 320,000<br>Positive |  |
| <p><u>Monkey # 127</u></p> <p>Immunization<br/>Dose, mg. (Sq)<br/>Reaction<br/>Antibody Titer<br/>Hemagglutination<br/>Gel-Diffusion<br/>Intravenous Challenge<br/>Dose (mcg./kg.)<br/>Reaction</p>   | 0.10<br>I    | 10,000<br>No Zone   | 0.25<br>N.R. | 320,000<br>Positive | 0.25<br>N.R. | 80,000<br>Positive | 20,000<br>No Zone   |  |

\* I, Illness (emesis, diarrhea)  
X, Death  
N.R. No Reaction

Date

|  | 1/8/64       | 1/31/64             | 2/12/64      | 3/12/64             | 3/18/64      | 4/1/64             | 6/18/64            |  |
|--|--------------|---------------------|--------------|---------------------|--------------|--------------------|--------------------|--|
|  |              |                     |              |                     |              |                    |                    |  |
| <u>Monkey # 128</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>N.R. | 20,000<br>Positive  | 0.25<br>N.R. | 320,000<br>Positive | 0.25<br>N.R. | 320,000<br>No Zone | 320,000<br>No Zone |  |
| <u>Monkey # 129</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>I    | < 10,000<br>No Zone | 0.25<br>N.R. | 160,000<br>Positive | 0.25<br>N.R. | 80,000<br>Positive | 40,000<br>No Zone  |  |

\* I, Illness (emesis, diarrhea)  
X, Death

N.R. No Reaction

Sample #26

Exposure to ultraviolet light for 1 hour

Date

|   | 1/8/64       | 1/31/64             | 2/12/64      | 3/12/64             | 3/18/64      | 4/1/64             | 6/18/64             |  |
|---|--------------|---------------------|--------------|---------------------|--------------|--------------------|---------------------|--|
| <u>Monkey #132</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>N.R. | < 10,000<br>No Zone | 0.25<br>N.R. | 80,000<br>No Zone   | 0.25<br>N.R. | 80,000<br>Positive | < 10,000<br>No Zone |  |
|   | 0.10<br>N.R. | < 10,000<br>No Zone | 0.25<br>N.R. | 320,000<br>Positive | 0.25<br>N.R. | 320,000<br>275     | 320,000<br>Positive |  |
| <u>Monkey #133</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>N.R. | < 10,000<br>No Zone | 0.25<br>N.R. | 320,000<br>Positive | 0.25<br>N.R. | 320,000<br>275     | 320,000<br>Positive |  |
|   | 0.10<br>N.R. | < 10,000<br>No Zone | 0.25<br>N.R. | 320,000<br>Positive | 0.25<br>N.R. | 320,000<br>275     | 320,000<br>Positive |  |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

Exposure to ultraviolet light for 1 hour

Date

|  | 1/8/64       | 1/11/64             | 2/12/64      | 3/12/64            | 3/18/64      | 4/1/64              | 6/18/64             |  |
|--|--------------|---------------------|--------------|--------------------|--------------|---------------------|---------------------|--|
| <b>Monkey # 134</b><br>Immunization<br>Dose, mg. (SQ)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I    | 20,000<br>No Zone   | 0.25<br>N.R. | 80,000<br>Positive | 0.25<br>N.R. | 40,000<br>Positive  | 40,000<br>No Zone   |  |
| <b>Monkey # 135</b><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>N.R. | < 10,000<br>No Zone | 0.25<br>N.R. | 40,000<br>Positive | 0.25<br>N.R. | 160,000<br>Positive | 160,000<br>Positive |  |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

Sample #28

Photodynamic action of methylene blue - 1 hour

Date

|  | 1/7/64    | 1/31/64             | 2/11/64      | 3/11/64             | 3/17/64      | 3/31/64              | 6/17/64             |  |
|--|-----------|---------------------|--------------|---------------------|--------------|----------------------|---------------------|--|
| <u>Monkey # 110</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I | 10,000<br>No Zone   | 0.25<br>N.R. | 160,000<br>Positive | 0.25<br>N.R. | 1,280,000<br>No Zone | 160,000<br>Positive |  |
|  | 0.10<br>I | < 10,000<br>No Zone | 0.25<br>N.R. | 80,000<br>Positive  | 0.25<br>N.R. | 320,000<br>125       | 320,000<br>Positive |  |
| <u>Monkey # 111</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>I | < 10,000<br>No Zone | 0.25<br>N.R. | 80,000<br>Positive  | 0.25<br>N.R. | 320,000<br>125       | 320,000<br>Positive |  |
|  | 0.10<br>I | < 10,000<br>No Zone | 0.25<br>N.R. | 80,000<br>Positive  | 0.25<br>N.R. | 320,000<br>125       | 320,000<br>Positive |  |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction



Photodynamic action of methylene blue - 1 hour

Date

|  | 1/7/64    | 1/31/64             | 2/11/64      | 3/11/64             | 3/17/64      | 3/31/64        | 6/17/64             |  |  |
|--|-----------|---------------------|--------------|---------------------|--------------|----------------|---------------------|--|--|
| <u>Monkey # 112</u><br>Immunization<br>Dose, mg. (sq)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>I | < 10,000<br>No Zone | 0.25<br>N.R. | 320,000<br>Positive | 0.25<br>N.R. | 320,000<br>165 | 80,000<br>Positive  |  |  |
|  | 0.10<br>I | 80,000<br>Positive  | 0.25<br>N.R. | 160,000<br>Positive | 0.25<br>N.R. | 640,000<br>150 | 320,000<br>Positive |  |  |
| <u>Monkey # 113</u><br>Immunization<br>Dose, mg. (sq)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>I | 80,000<br>Positive  | 0.25<br>N.R. | 160,000<br>Positive | 0.25<br>N.R. | 640,000<br>150 | 320,000<br>Positive |  |  |
|  | 0.10<br>I | 80,000<br>Positive  | 0.25<br>N.R. | 160,000<br>Positive | 0.25<br>N.R. | 640,000<br>150 | 320,000<br>Positive |  |  |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

Sample #28

Photodynamic action of methylene blue - 1 hour

Date

|  | 1/7/64       | 1/31/64            | 2/11/64      | 3/11/64               | 3/17/64      | 3/31/64          | 6/17/64             |  |
|--|--------------|--------------------|--------------|-----------------------|--------------|------------------|---------------------|--|
| <u>Monkey # 114</u><br>Immunization<br>Dose, mg. (Sq)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>N.R. | 50,000<br>Positive | 0.25<br>N.R. | 1,280,000<br>Positive | 0.25<br>N.R. | 1,280,000<br>115 | 160,000<br>Positive |  |
| <u>Monkey # 115</u><br>Immunization<br>Dose, mg. (Sq)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>I    | 10,000<br>No Zone  | 0.25<br>N.R. | 80,000<br>Positive    | 0.25<br>N.R. | 640,000<br>115   | 320,000<br>Positive |  |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

Photodynamic action of methylene blue - 1 hour

Date

|  | 1/7/54    | 1/31/54            | 2/11/54      | 3/11/54             | 3/17/54      | 3/31/54               | 6/17/54             |  |
|--|-----------|--------------------|--------------|---------------------|--------------|-----------------------|---------------------|--|
|  | 0.10<br>I | 10,000<br>Positive | 0.25<br>N.R. | 320,000<br>Positive | 0.25<br>N.R. | 1,280,000<br>Positive | 320,000<br>No Zone  |  |
| <u>Monkey # 116</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction |           |                    |              |                     |              |                       |                     |  |
|  | 0.10<br>I | 80,000<br>200      | 0.25<br>N.R. | 640,000<br>Positive | 0.25<br>N.R. | 640,000<br>140        | 640,000<br>Positive |  |
| <u>Monkey # 117</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   |           |                    |              |                     |              |                       |                     |  |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction

Sample #28

Photodynamic action of methylene blue - 1 hour

Date

| <p>Monkey # 11E</p> <p>Immunization<br/>Dose, mg. (Sq)<br/>Reaction *<br/>Antibody Titer<br/>Hemagglutination<br/>Gel-Diffusion<br/>Intravenous Challenge<br/>Dose (mcg./kg.)<br/>Reaction</p> | 1/7/54       | 1/31/54            | 2/11/54      | 3/11/54               | 3/17/54      | 3/31/54            | 6/17/64             |  |
|--|--------------|--------------------|--------------|-----------------------|--------------|--------------------|---------------------|--|
|  | 0.10<br>N.R. | 10,000<br>Positive | 0.25<br>N.R. | 1,280,000<br>Positive | 0.25<br>N.R. | 150,000<br>500     | 640,000<br>Positive |  |
| <p>Monkey # 11F</p> <p>Immunization<br/>Dose, mg. (Sq)<br/>Reaction<br/>Antibody Titer<br/>Hemagglutination<br/>Gel-Diffusion<br/>Intravenous Challenge<br/>Dose (mcg./kg.)<br/>Reaction</p>   | 0.10<br>I    | 50,000<br>No Zone  | 0.25<br>N.R. | 150,000<br>Positive   | 0.25<br>N.R. | 30,000<br>Positive | 320,000<br>Positive |  |
|  |              |                    |              |                       |              |                    |                     |  |

\* I, Illness (emesis, diarrhea)  
X, Death  
N.R. No Reaction

Photodynamic action of methylene blue - 1 hour

Date

|  | 1/7/64    | 1/31/64           | 2/11/64      | 3/11/64             | 3/17/64      | 3/31/64           | 6/17/64            |  |
|--|-----------|-------------------|--------------|---------------------|--------------|-------------------|--------------------|--|
| <u>Monkey # 120</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction *<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction | 0.10<br>X |                   |              |                     |              |                   |                    |  |
| <u>Monkey # 121</u><br>Immunization<br>Dose, mg. (SQ)<br>Reaction<br>Antibody Titer<br>Hemagglutination<br>Gel-Diffusion<br>Intravenous Challenge<br>Dose (mcg./kg.)<br>Reaction   | 0.10<br>I | 40,000<br>No Zone | 0.25<br>N.R. | 160,000<br>Positive | 0.25<br>N.R. | 80,000<br>No Zone | 160,000<br>No Zone |  |

\* I, Illness (emesis, diarrhea)  
 X, Death  
 N.R. No Reaction